Study of ripe *Rhizophora mucronata* fruit flour as functional food for antidiabetic

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**Abstract**

Ripe *Rhizophora mucronata* has been consumed by mangrove society in Indonesia. The aim of the research was to study the potency of ripe *R. mucronata* fruit flour, reducing blood glucose level in Alloxan-induced diabetic rats. Ripe *R. mucronata* fruit flour was administrated to Alloxan (125 mg/kg body weight)-induced diabetic male Wistar rats at different doses (500, 1000, 1500 and 2000 mg/day/head) for 18 days and a positive control group given standard drug for diabetic (glibenclamide 0.09 mg/day/200 g body weight). The result showed blood glucose level of negative control groups increased, whereas positive control group and experimental rats group fed flour of mangrove fruit significantly declined. Ripe of mangrove fruit flour at doses 1000, 1500, 2000 mg/day/head body weight indicated similar effect to glibenclamid in decreasing blood glucose level. The potency of ripe *R. mucronata* fruit flour as antidiabetic related to the phytochemical compounds of its such as tannins, saponins, flavonoids, and steroid. Ripe *R. mucronata* fruit flour contained 7.50% soluble dietary fiber and 38.60% insoluble dietary fiber. Thus the ripe *R. mucronata* fruit flour is a candidate as functional food source especially antidiabetic.

**Keywords**

Antidiabetic
Dietary fiber
Phytochemical
Mangrove fruit flour

**Introduction**

Mangroves are halophytes plants lived between terrestrial and sea (Giri *et al.*, 2010). Mangroves have exploited as a source of timber, fuel, food, and medicine. Extracts and chemical components of mangroves has been used as insecticides and pesticides, tannins for the leather industry and for dying, substitute for salt, potassium carbonate and sodium chloride, tonic, wine and a fruit drink, betel substitute, and vegetable (Bandaranayake, 1998; Alongi, 2002). Mangrove plants is utilized as food and medicine from the roots, stems, leaves, flowers and fruit. Utilization was related to the content of nutrients (proteins, fats, carbohydrates, vitamins, and minerals) and bioactive compounds in mangrove plant (Bandaranayake, 1998).

Chemical compounds and bioactive of mangrove plants can act as antimicrobial (Choudhury *et al.*, 2005; Agaramoorthy *et al.*, 2007; Sivaperumal *et al.*, 2010; Abeyesinghe, 2012; Dhayanithi *et al.* 2012), anti-cancer (Prabbu and Guruvayoorappan, 2011), antioxidants (Bamarjee *et al.*, 2008; Vadlapudi and Naidu, 2009; Wei *et al.*, 2010; Abdullah *et al.*, 2013), anti inflammation (Hossain *et al.*, 2011), and others associated with drugs and food. Moreover, the mangrove plants containing bioactive are used as food, so the food can be stated as a functional food.

One species of mangrove plants in Indonesia used as medicine and food are *Rhizophora*. *Rhizophora* leaves cure diarrhea traditionally and the fruit consumed by people around the mangrove. This is supported by Bandaranayake (1998) the traditional parts of plants of various types *Rhizophora* used to treat various types of diseases. *R. apiculata* is an antiemetic, antiseptic, diarrhea, haemostatic, (bark), hepatitis, (bark, flowers, fruit, leaves), stops bleeding, typhoid, (bark). *R. lamarckii* as hepatitis drug, (flowers, leaves). *R. mangle* is angina, boils and fungal infections, (bark), antiseptic, diabetes, dysentery, elephantiasis, fever, malaria, leprosy, (bark, leaves), minor bruises, (bark), plaster for fractured bones, (bark), tuberculosis (bark, leaves). *R. mucronata* as elephantiasis drug, febrifuge, hemotoma, (bark), hepatitis, (bark, flowers, fruit, leaves, roots), ulcers, (bark). *R. racemosa* is the bleeding stops, (flowers, leaves). *Scaevola sericea* is a medicinal antiseptic, anti-inflammatory, coughs, diabetes, eye infections, gastro-intestinal disorders, headaches, stings and bites, (bark, leaves).

The part of mangrove plants used as food in Indonesia is a fruit. The fruit is dried and made into flour that it can be used for a wide variety of food products. Meanwhile, mangrove plants contain bioactive such as tannins, alkaloids, steroids, anthroquinone glycosides, and flavonoids (Patra *et al.*, 2009). Odom *et al.* (2013) was reported that some fruit powder mixture containing alkaloids, tannins, saponins and...
flavonoid have hypoglicemic (antidiabetic) activity in Alloxan-induced diabetic rats. *R. mucronata* contains polyphenols 157.4±22.9 mg/g dw and free radical scavenging activity 83.7±2.8 mg/mL (Agoramoorthy et al., 2008), Rhizophorins CE (1-3), Rhizophorin A, (6 R, 11 S, 13 S) -6,11,13-trihydroxy-2 ,3-seco-14-labden-2 ,8-olid-3-oic acid and Rhizophorin B, ent-3β ,20-epoxy-3,18-dihydroxy-15-beyerene and unknown activities (Ammanamanchi, 2004) and triterpenoids: β-amyrin, lupeol and tarataxol (Basyuni, 2008). In addition, it was reported also that of mangrove leaves and pneumatophore of *Ceriops decandra* have antinoceptive and antidiabetic activity (Uddin et al., 2005; Mannalamkunnath, 2010). The extract of *R. mucronata* bark had the highest α-glucosidase inhibitory activity with IC$_{50}$ at 0.08±1.82 μg mL$^{-1}$ so it can be an candidate of antidiabetic (Lawag et al., 2012).

Bunyapraphatsara et al. (2002) reported that *R. mucronata* contain dietary fiber (29.25% ± 0.4%). Meyer et al.,(2000) reported that dietary fiber from whole grains are protective against the development of diabetes. Furthermore, Jenkins et al. (2000) stated that water soluble dietary fiber is protective against diabetes than insoluble dietary fiber. Chandalia et al. (2000) suggest that the consumption of dietary fiber on the ADA recommended especially water soluble will improve control of blood sugar, lowering hyperinsulinemia, and lowering plasma lipid concentrations of patients with type 2 diabetes.

Due to the phytochemical content on mangrove plants and food fiber content is high enough in the mangrove *R. mucronata*, it is necessary to study the role of mangrove *R. mucronata* flour fruit consumed in Indonesia as hypoglycemia or antidiabetic activity.

Material and Methods

Plant preparation

Materials studied were *R. mucronata* mangrove fruit flour made from ripe fruit harvested from mangrove areas Penunggul village, Pasuruan, East Java, Indonesia. Mangroves ripe fruit marked by a yellow line on the hypocotyl.

Flour-making process begins with the cutting of fruit with a length of about 10 cm, peeled and soaked in water. Furthermore, drained fruit and soaked in a solution of 0.5% citric acid for 10 minutes, and followed by immersion in water for 3 days. After that, the fruit drained, reduced in size and dried using an oven at a temperature of 75°C for 3 hours or until the moisture content of the material below the 14.5% (Lidiasari et al., 2006). The dried fruit pieces milled by dissmiller and sieved using a 60 mesh siever, in order to obtain fruit flour *R. mucronata*. The flour in the proximate analysis, levels of dietary fiber enzymatically (AOAC, 1995), levels of tannins (Ranganna, 1986), the levels of HCN (Baskin and Brewer, 2006), phytochemical test (Harborne, 1987), hypoglycemic activity, and toxicity (Meyer et al., 1982).

Animals

Experimental animal models used were male Wistar rats, aged 2.5-3.0 months, and 150-200 g body weight. Initially rats was adapted for seven days with standard feed. Diabetes was induced by an intraperitonial injection of 125 mg/kg body weight. After alloxan injection, the diabetic rats (glucose level >125 mg/dL) were used for the study.

Hypoglycemic effect assay

Furthermore, diabetic rats were divided into 6 groups (each consist of 5 rats). The group given feed containing 500 mg/day/head, 1000 mg/day/head, 1500 mg/day/head, 2000 mg/day/head ripe *R. mucronata* fruit flour, the standard feed group (negative control), and positive control group (standard feed and drug glibenclamide at a dose of 0.09 mg/day/200 g body weight). The experiment was conducted over 18 days with a feeding system feeding level and provision of drinking at libitum. Measurement of blood glucose levels every 3 days during 18 days. In addition, the measurement both of body weight and feces was conducted every day.

Measurement of blood glucose levels

Measurement of blood glucose levels of rats uses GOD-PAP enzymatic method. It was analyzed by kit glucose GOD FS (Dyasys product of Dyasys Diagnostic Systems GmbH&Co. KG Germany). GOD-PAP method in principle is the oxidation of glucose by Gluko-oxidase (GOD) into gluconic acid and H$_2$O$_2$. Furthermore, H$_2$O$_2$ reacted with 4-aminontipirin and phenol which produces chinonimine are colored reddish and H$_2$O. This reaction is catalyzed by the enzyme peroxidase (POD). Chinimine formed equivalence with glucose so that the measured color of chinimine be proportional to glucose levels.

At first, the rats were fasted for 12 hours beforehand and then have blood drawn (approximately 1 ml) through the orbital sinus using microhematokrit and put in eppendorf tubes. Blood settling 30 minutes at room temperature to clot, then centrifuged blood for 15 minutes at 3000 rpm (up to form 2 layers). The top layer is clear yellow color, taken with eppendorf pipette and inserted to measure blood glucose levels.
Glucose + O₂ $\xrightarrow{GOD}$ Glukonic acid + H₂O₂

2H₂O₂ + 4 – aminoantipirin + phenol $\xrightarrow{POD}$ Chinonimine + H₂O

Reagents used consisted of a phosphate buffer 50 mmol/l, pH 7.5 glucose oxidase > 10 KU/I, phenol 5 mmol/l, peroxidase > 10 KU/I and 4 aminoantipirin 0.5 mmol/l. The standards used were glucose 100 mg/dl (= 5.55 mmol/l). Solution samples / standards were made by mixing 10μl reagent reagent. As for the blank solution consisting only of 1000 μl reagent. These solutions are then settling at room temperature (26°C) for 20 minutes or for 10 minutes at 37°C. Absorption is read by colorimetry at λ 500 nm or 546 nm. Solution is used as the zero point blank. Glucose levels in the blood is calculated by the following formula:

$$\text{Blood glucose (mg/dL)} = \frac{\text{As}}{\text{Ast}} \times \text{standard content}$$

Note:
- As : The absorbance of the sample
- Ast : The absorbance of the standard

Measurement of urine glucose

Urine glucose was measured by uriscan kit (Urine Scan Glucose product of YD Diagnostics). 1 ml urine of rats is filled into appendorf. Then, uriscan inserted into appendorf for one minute or until the colour changes. Uriscan was removed and dried. The colour shown in uriscan will indicate the value or condition of rat urine glucose test.

Toxicity tests with Brine Shrimp Lethal Test

Toxicity tests using Artemia salina shrimp larvae as test animals (Meyer et al., 1982). A. salina eggs (Artemia) incubated in artificial sea water (38 grams of salt in 1000 mL of distilled water) under a 15-watt fluorescent lamp. After 48 hours, the eggs hatch into nauplii instar III/IV and ready to be used as test animals. Subsequently made a series of concentration of methanol extract of R. mucronata flour amounted to 156, 312, 625, 1250, 2500, 5000 and 10,000 ppm of stock solution with saline diluent. Used as a control solution of 0.8% DMSO in physiological saline solution without the extract. Ten larvae A. salina inserted into the vial containing the sample extract in different concentrations series. Each treatment and control replicates performed 3 times. A. salina maximum larval mortality in the control should not exceed 10%. Of the total population of the test. Furthermore, all the vials were incubated under a fluorescent lamp of 15 watts for 24 hours. After incubation, the number of A. salina larvae that died in each vial was calculated to determine the percentage of death. Toxicity effects were analyzed from observations with the percent mortality.

$$\% \text{ larva} = \frac{\text{Total larvae death}}{\text{Total larvae test}} \times 100\%$$

By knowing the mortality of larvae Artemia salina, and then look through the tables and figures made probit equation:

$$Y = Bx + A$$
$$Y = \log \text{concentration, and}$$
$$X = \text{probit value}$$

From equation is then calculated by entering values probit LC₅₀ (50% mortality). If the existing control larvae were dead, then death is determined by the formula % Abbot (Meyer et al., 1982).

Result and Discussion

Chemical characteristic of ripe R. mucronata fruit flour

Chemical characteristic of ripe R. mucronata fruit flour based on the proximate analysis, tannin content and dietary fiber are shown on Table 1. In addition, phytochemical analysis of ripe R. mucronata fruit flour was measured qualitatively (Table 1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ripe R. mucronata fruit flour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (%)</td>
<td>3.50</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>0.78</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>2.90</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>1.27</td>
</tr>
<tr>
<td>Carbohydrate (by diff) (%)</td>
<td>90.67</td>
</tr>
<tr>
<td>HCN (ppm)</td>
<td>2.97</td>
</tr>
<tr>
<td>Tannin (ppm)</td>
<td>819</td>
</tr>
<tr>
<td>Dietary Fiber : Insoluble (%)</td>
<td>38.6</td>
</tr>
<tr>
<td>Soluble (%)</td>
<td>7.50</td>
</tr>
<tr>
<td>Total (%)</td>
<td>46.10</td>
</tr>
<tr>
<td>Colour</td>
<td>Light brown</td>
</tr>
<tr>
<td>Yield (%)</td>
<td>12.9</td>
</tr>
<tr>
<td>LC₅₀ (BSLT)(ppm)</td>
<td>1737.80</td>
</tr>
</tbody>
</table>

According to the chemical composition of ripe R. mucronata fruit flour, it was dominate by carbohydrate (90.67%), low-protein, and low-fat.
Carbohydrate contained high dietary fiber consisted of 38.60% insoluble dietary fiber and 7.50% soluble dietary fiber. Thus, ripe R. mucronata fruit flour had catagorize as high-dietary fiber. Dietary fiber of ripe R. mucronata fruit flour is higher than R. mucronata fruit (29.25±0.4%). Meyer et al. (2000) reported that dietary fiber from whole grain are protective toward diabetes. Soluble dietary fiber more protective than insoluble dietary fiber (Jenkins et al., 2000). Chandalia et al. (2000) stated that consumption of dietary fiber especially of the soluble type above the level improves blood glucose control, reduce hyperinsulinemia, and decreases of plasma lipid concentration in patient with type 2 diabetes. Level of food safety can be measured by HCN content, tannin content and LC50 value. Yield is comparison between the amount of ripe R. mucronata fruit flour and the amount of ripe R. mucronata fruit. Yield of flour was 12.9%. The fluctuation of yield was influenced by the amount of water and other component lost during processing.

Toxicity test of mangrove fruit flour
Meyer et al. (1982) and Effendi et al. (2012) grouped level of toxicity based on the LC50 value, into five groups: firstly, the substance had LC50 values < 1 ppm is expressed very toxic, 1-100 ppm is moderately toxic, 100-1000 ppm is nearly toxic, 1000-10,000 ppm is low toxic, 10,000-100,000 ppm is nearly non-toxic and over 100,000 ppm is declared not toxic. The LC50 value of ripe R. mucronata fruit flour was 1737.80 ppm, it was grouped into low toxic levels. The low level of toxicity is assumed related to both of HCN (2.97 ppm) and tannin (819 ppm). The safe limit of HCN in the diet is 50 ppm. Moreover the safe limit of tannin in the diet is 560 mg/kg body weight/day.

Phytochemical screening
Phytochemical screening of ripe R. mucronata fruit flour showed the presence of tannin, saponin, flavonoid, and steroid. There was a slight different reported of Ghosh et al. (1985) that R. mucronata contained steroid, triterpenoid, alkaloid, flavonoid, tannin, catechin, quinon and antocyanidin. The content of phytochemical role as bioactive. Patra et al. (2009) and Odom et al. (2013) reported that the mixed of some fruits containing alkaloid, tannin, saponin and flavonoid have hypoglicemic activity (anti diabetic) toward rat induced alloxan.

Hypoglycaemic effect of ripe R. mucronata fruit flour
The blood glucose level of experimental rats was administrated ripe R. mucronata fruit flour at different doses (Figure 1). Ripe R. mucronata fruit flour reduced blood glucose of male Wistar rats providing that compared to the negative control. The higher a dose was administrated, the higher the effect to diminish blood sugar level in diabetic rats. The result suggested that ripe R. mucronata fruit flour possesses hypoglycaemic activities and it could be used as a functional food for diabet.

The potency of ripe R. mucronata fruit flour in reducing the blood glucose level related to the content both of dietary fiber (46.10%) and bioactive, as antidiabetic. Meyer et al. (2000) reported that dietary fiber from whole grain are protective toward diabetes. Soluble dietary fiber more protective than insoluble dietary fiber (Jenkins et al., 2000). Chandalia et al. (2000) stated that consumption of dietary fiber recommended by ADA especially soluble type will increase blood glucose control, reduce hyperinsulinemia, and decrease of plasma lipid concentration in patient with type 2 diabetes. According to Dianitami (2009), dietary fiber has physiological effects such as reduce transit time canals digestion, slows gastric emptying, prolong satiety, beneficial intestinal flora to normal, increase the secretion of the pancreas, increases the production of short chain fatty acids, increasing the levels of serum lipids and bile acid binding. Fiber slows the gastric emptying to prevent increasing the level of blood glucose. Food will be released into small intestine and it blood glucose levels rise slowly.

The role of bioactive phytochemicals as antidiabetic was reported by Patra et al. (2009) and Odom et al. (2013) that the mixture of some fruit powder containing alkaloids, tannins, saponins and flavonoid have hipoglicemic activity (antidiabetic) in alloxan-induced diabetic rats. The leaf powder of Rhizophora are a potent drug of antidiabetic activity because it contains an insulin-like protein. It also was on par with glibenclamide (Alikunhi et al., 2012).

Furthermore, the effect of mangrove fruit flour blood as antidiabetic can be compared with drug...
glibenclamide (positive control) by measuring the average blood sugar level (Figure 2). According to Davey (2005) and Katzung (2007), glibenclamide is one of sulfonylurea group enhancing insulin secretion by pancreatic beta cells.

From the histogram in Figure 2 can be seen that the higher the flour doses given, the decrease in blood glucose levels of rats. Based on the LSD test, oral administration of ripe \textit{R. mucronata} fruit flour doses 1000 mg/day/head, 1500 mg/day/head, and 2000 mg/day/head was not showed significantly effect, compared to positive control (glibenclamide 0.09 mg/day/200 g body weight) (p<0.05). Moreover, ripe \textit{R. mucronata} fruit flour 1000 mg/day/head decreased blood glucose in rats-induced alloxan, it was similar to the ability of the drug glibenclamide.

\textbf{Changes of body weight}

Based on the figure 2, the rate of decline of body weight was started on the day 3. Diabetic patient can not burn glucose so it burns fat. It lead to weight loss. Overall, during 18 days, the rate of body weight decreased. However, the administration of ripe \textit{R. mucronata} fruit flour 2000 mg/day/head, 1000 mg/day/head and positive control showed upward trends. The minimum of the rate body weight were at dose 500 mg/day/head, 1500 mg/day/head and negative control. The higher dose of the fruit flour related to the dietary fiber content of flour. Dietary fiber decrease blood glucose level by slowing the emptying of stomach, it prevent an upsurge of blood glucose level (Dianitami, 2009). The extract of \textit{R. mucronata} leaves and glibenclamide were maintained th body weight of Streptozotocine-induced diabetic rats, but the body weight of diabetic rats had gone down (Pandey et al., 2014).

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|}
\hline
\textbf{Treatment} & \multicolumn{9}{c|}{\textbf{Repetition}} \\
\hline
 & 0 & 3 & 6 & 9 & 12 & 15 & 18 \\
\hline
\textit{R. mucronata} flour & & & & & & & \\
500 mg/day & 1 & +++ & +++ & +++ & +++ & +++ & + \\
1000 mg/day & 2 & +++ & +++ & +++ & +++ & +++ & ++ \\
1500 mg/day & 3 & +++ & +++ & +++ & +++ & +++ & ++ \\
2000 mg/day & 3 & +++ & +++ & +++ & +++ & +++ & ++ \\
\hline
Negative control & 1 & +++ & +++ & +++ & +++ & +++ & +++ \\
Positive control & 2 & +++ & +++ & +++ & +++ & +++ & +++ \\
\hline
\end{tabular}
\caption{The urine glucose levels of rat}
\end{table}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2}
\caption{Response of consumption of ripe \textit{R. mucronata} fruit flour to the blood glucose level}
\end{figure}

\textbf{Urine glucose level}

Reduction of blood glucose level affect a significant decrease urine glucose (Table 2). The positive control group showed a normal urine glucose on day 12, whereas the administration of ripe \textit{R. mucronata} fruit flour 1000, 1500, and 2000 mg/day/head body weight normal urine glucose at day 15. It can be concluded that the ability of the fruit flour mangrove 1000 mg/day/head is similar to the
drug glibenclamide.

According to Suriani (2012), urine glucose levels related to the with excess blood sugar levels released through the urine. Sugar is filtered by the kidneys glomerulus continuously but it will be returned to the blood system through a system of blood flow renal tubular reabsorption. Limited capacity of the kidneys reabsorbing glucose was a rate of 350 mg/min. When glucose levels are very high, glomerulus filtrate containing glucose above the threshold for reabsorbed. As a result, the excess glucose is excreted through the urine. This phenomenon is called glycosuria, which is another indication of diabetes mellitus.

Conclusion

Ripe \textit{R. mucronata} fruit flour is dominated by carbohydrate 90.67% contained 7.50% soluble dietary fiber and 38.60% insoluble dietary fiber. Both of HCN and tannins content of ripe \textit{R. mucronata} fruit flour is under maximum level to consume and low toxic. Ripe \textit{R. mucronata} fruit flour qualitatively mangrove fruit contains phytochemical compounds such as flavonoids, steroids, saponins and tannins. Ripe \textit{R. mucronata} fruit flour reduced blood glucose level in diabetic rats. Dose of 1000 mg/day/head, 1500mg/day/head, and 2000 mg/day/head have the similar to activity of glibenclamide (0.09 mg/day/200g body weight) in lowering blood glucose diabetic rats.

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References


