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Research Article

THE EFFECT OF HEATING TEMPERATURE ON INHIBITORY ACTIVITY OF MANGROVE *RHIZOPHORA MUCRONATA* FRUIT EXTRACT TOWARD A-GLUCOSIDASE

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ABSTRACT

Objectives: The aim of this research was to determine the effect of heating temperature of mangrove *Rhizophora mucronata* fruit extract toward its inhibition activity of α-glucosidase.

Methods: Research method used was an experimental method with the treatment of heating temperature (70°C, 80°C, 90°C, and 100°C) of crude tannin extract of mangrove *R. mucronata* fruit on inhibition activity toward α-glucosidase.

Results: IC_{50} of unheated extract and extracts that were heated at 70°C, 80°C, 90°C, and 100°C are adalah 3.38 ppm, 2.79 ppm, 2.97 ppm, 3.19 ppm, and 3.52 ppm, respectively. Heating until 100°C only decreased its inhibition activity of α-glucosidase of about 3.87% compared to unheated extract. Mangrove *R. mucronata* fruit extract contains phytochemical compounds, i.e., alkaloids, saponin, flavonoids, tannin, and terpenoids. IC_{50} of this extract is lower compared to IC_{50} of acarbose (antidiabetic medicine), which as IC_{50} value of 13.27 ppm.

Conclusion: Mangrove *R. mucronata* fruit extract is quite heat resistant and is potential to be an antidiabetic functional food.

Keywords: *Rhizophora mucronata* fruit extract, IC_{50}, Heating temperature.

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INTRODUCTION

Diabetes mellitus is glucose metabolic disorder caused by the decrease in insulin effectiveness. The decrease in insulin secretion causes an increase in blood glucose level [1]. Increase in blood glucose level is also related to α-glucosidase enzyme activity in intestine in carbohydrate digestion and glucose absorption by epithelium [2]. One of the methods to decrease blood glucose level on diabetes mellitus patient is by inhibiting α-glucosidase enzyme and its activity on small intestine [3]. There are several *in vitro* researches that used a combination of α-amylase and α-glucosidase enzymes on various materials [4,5]. Inhibition toward α-glucosidase is effective in decreasing glucose digestion and absorption in small intestine. Thus, it can lower the postprandial blood glucose level on diabetes mellitus patients [6]. Synthetic inhibitory agent in diabetes mellitus treatment is done by consuming acarbose and miglitol (diabetic medicine), but these synthetic inhibitory agents may cause side effects and increase the diabetes complication. Therefore, based on several experiments and scientific research of bioactive compounds, medicinal drugs from herbal sources are comparable to synthetic medicine [7]. Herbal products, both in forms of pure compounds or extract, have a chance to be developed in the area of medicine [8].

Since mangroves usually inhabit the transition zone between land and sea, it is expected that mangroves would be able to produce many natural products from their own secondary metabolites [9]. One of mangrove plants from *Rhizophora mucronata* type has secondary metabolites which are potential to become antidiabetic agents. Fruit part of black mangrove *R. mucronata* Lam has secondary metabolites, i.e., flavonoids, saponin, phenolic, hydroquinone, and tannins [10]. Crude extract of *R. mucronata* fruit was reported to be able to inhibit the activity of α-glucosidase enzyme, and the extract contained flavonoids, saponin, steroid, and tannins [11]. It was also reported that fruit from *R. mucronata* in the forms of powder could cure the rats with type 2 diabetes [12]. Tannins are the main compounds in mangrove *R. mucronata*; therefore, it is suspected that tannins play an important role as an antidiabetic agent. Moreover, it was also reported that tannins are polyphenolic compounds that can also act as an antioxidant [13]. There is also a correlation between polyphenol and antioxidant and antidiabetic activity, such as in tea [14,15].

Phenolic antioxidant compounds that are relatively high can be considered as antidiabetic drugs [16]. The activity and composition of phenolic compounds and tannins are influenced by heating temperature. Heat processing could influence the stability of phenolic antioxidant compounds [17]. It is because generally antioxidant has a heat-labile property [18]. Study about the effect of temperature and pH is commonly done to determine the activity, stability and/or characteristics of certain compounds, e.g., antioxidant activity of leek [19], antioxidant activity of rice bran [20], characteristics of tannin extract from Gambier [21], characteristics of color compound and tannin from guava leaves [22,23], and enzyme and flavonoid antimicrobe [24]. Besides, the process to obtain bioactive compounds is commonly done using heat and pH. Therefore, it is required to do research to determine the effect of heating temperature toward inhibitory activity of *R. mucronata* extract toward α-glucosidase enzyme.

RESEARCH METHODS

Materials and equipment

Materials used in this research consisted of materials for preparation of ripe mangrove *R. mucronata* fruit powder, extraction of ripe fruit from *R. mucronata*, characterization of temperature, total phenolic content, total tannin content, and total condensed tannin content and determination of inhibition activity toward α-glucosidase. Materials used for the making of ripe mangrove *R. mucronata* fruit powder included ripe fruit of mangrove *R. mucronata* and 0.5% citric acid (Merck, Germany). Materials used for extraction of ripe mangrove

R. mucronata fruit and temperature characterization were 70% acetone p.a. (v/v), 0.25% ascorbic acid (Merck), aquadest, filter paper, aluminum foil, and dimethyl sulfoxide (DMSO). Materials used for the determination of total phenolic content, total tannin, and total condensed tannin were extract from ripe fruit *R. mucronata*, without temperature characterization (as a control) and with temperature characterization (70°C, 80°C, 90°C, and 100°C), Folin–ciocalteu, 7.5% Na_2CO_3 (w/v), polyvinylpyrrolidone (PVPP), butanol-HCl (95:5), ferric reagent, and aquadest. Materials used for inhibitory activity assay toward α-glucosidase were ripe mangrove *R. mucronata* fruit extract, 0.1 ml α-glucosidase (Megazyme), substrate p-4-Nitrophenyl β-D-glucopyranoside (PNPG) (Sisco Research Ltd.), DMSO and buffer pH, 200 mMNa_2CO_3 (Merck), Bovine Serum Albumin (BSA) (Calbiochem), and K_2HPO_4.

Methods

The research method used was experimental methods with heating temperature of mangrove fruit extract as a treatment used to determine its effect on inhibitory activity toward α-glucosidase. The temperature used was 70°C (A_1), 80°C (A_2), 90°C (A_3), and 100°C (A_4), also extract without any heating treatment (A_0). Heat treatment was applied on the extract after it was dissolved in DMSO solvent with ratio of 1:10 (w/v) and heat was applied for about 10 min [25].

Preparation of mangrove *R. mucronata* fruit powder

Main material used in the preparation of powder was mangrove *R. mucronata* ripe fruit. Ripe fruits were obtained from Nguling, Pasuruan district, East Java with characteristics as follows: The cotyledon has yellow color and the fruit has darker color. Fruit powder making started with cleaning and cutting the fruits to obtain 10 cm length and soaking them in 0.5% citric acid solution for 10 min, draining and followed by blanching in hot water for 10 min. After blanching, the fruits were then soaked in water for 3 days, drained and sun-dried for 2 days. After drying, mangrove fruit pieces were then milled using disc mill and sieved using 80 mesh sieve to obtain mangrove fruit powder [12].

Extraction and temperature treatment of extract

Extraction process was done based on the method by Zhou *et al*. [26], using sonicator with 40% amplitude. First, 25 g of mangrove fruit powder was dissolved using 70% acetone-water (v/v) and added with 0.25% ascorbic acid. This mixture was then sonicated for 30 min and centrifuged with 3000 rpm speed for 10 min to obtain a supernatant as tannin crude extract. This crude extract was then heated using temperature of 70, 80, 90, and 100°C for 10 min. The observed parameters consist of yield, inhibition toward α-glucosidase [27], total phenolic content [28], and total tannins [29].

Inhibitory activity toward α-glucosidase assay

In vitro inhibition assay requires several materials, i.e., α-glucosidase (Megazyme), substrate p-4-Nitrophenyl β-D-glucopyranoside (PNPG) (Sisco Research Ltd.), DMSO (Merck), 200 mM Na_2CO_3 (Merck), BSA (Calbiochem), and acarbose (PT. Bayer, Indonesia). Inhibitory activity toward α-glucosidase was determined by enzymatic mixing reaction, consists of 10 µL of sample in DMSO, 490 µL phosphate buffer (pH 7), and 250 µL of 20 Mm PNPG substrate, and homogenized using vortex. The mixture was then incubated at 37°C for 5 min. Then, 250 µL of α-glucosidase solution was added (as C and S_1), and 250 µL of phosphate buffer was also added (as B and S_0). Then, the mixture was incubated again at 37°C for 15 min. The enzymatic reaction was stopped by adding 1000 µL of 200 mM sodium carbonate. This reaction would produce p-nitrophenol which has yellow color. The p-nitrophenol obtained from this reaction was then measured for its absorbance at 400 nm wavelength [27]. The enzymatic mixing reaction of α-glucosidase can be observed in Table 1.

Percentage inhibition is calculated using formula: {(C-S)/C}×100%, where C= absorbance of control (DMSO) without sample (C-B) and S = absorbance of sample (S_1-S_0).

Acarbose solution was used as positive control. Acarbose solution was prepared by dissolving acarbose in buffer and 2 N HCl (1:1) with concentration of 1% (b/v). The solution was centrifuged and the supernatant was used as a standard or positive control. 10 µL of supernatant was taken and put inside the reaction mixture, such as in extract sample. Concentration of *R. mucronata* fruit extract used was 6.25 ppm, 12.50 ppm, 25.00 ppm, and 50.00 ppm (w/v) in DMSO solution.

Determination of total tannin content

Total tannins were determined using reaction between tannin compound and PVPP. About 100 mg of PVPP was put inside reaction tube and added with 1 mL of aquadest and 1 mL of *R. mucronata* fruit extract, homogenized using vortex and incubated at a temperature of 4°C for 15 min. The mixture was then homogenized using vortex and centrifuged (3000 rpm for 10 min). The obtained supernatant was used as total non-tannin phenolic. Total tannins were calculated using the formula: Total tannins = total phenolic - total non-tannin phenolic [28].

RESULTS AND DISCUSSION

Yield and phytochemical content

Yield is percentage obtained from the ratio between final weight and initial weight of sample, multiplied by 100%. Yield calculation is used to determine the percentage of the extractable part from a raw material [16]. Ripe fruit from mangrove *R. mucronata* was made into powder before extraction process to increase the extraction rate and effectiveness. Characteristics of mangrove fruit powder obtained are brown color and fibrous. The fibers were then discarded using 60 mesh sieves to decrease the particle size of the raw material to ease the extraction process.

The increase in extraction effectiveness is influenced by size of material used, in which the smaller the particle size, the higher the surface area between the material and its solvent [30]. The yield data of ripe fruit *R. mucronata* crude extract and powder can be observed in Table 2. Qualitative analysis of crude tannin extract from mangrove leaves contain alkaloids, flavonoids, terpenoid, tannins, and saponin (Table 3). It is similar to results from crude methanol extract of black mangrove leaves [31], but in crude extract, tannins compound was more dominant.

Table 3 shows that crude extract of *R. mucronata* fruits contains secondary metabolites, such as alkaloids, flavonoids, tannins, saponin, and triterpenoid, but it does not contain steroid. In correlation with blood glucose, triterpenoids, and saponin have been reported to be able to act as antibacterial, anti-inflammatory, antibiotics, hemolytic drug,

Table 1: Enzymatic mixing reaction system of α-glucosidase

Material	Blank (µL)	Control (µL)	S_0 (µL)	S_1 (µL)
Sample	-	-	10	10
DMSO	10	10	-	-
Phosphate buffer pH 7	490	490	490	490
Substrate	250	250	250	250
Incubated in water bath (37°C for 5 min)				
Phosphate buffer pH 7	250	-	250	-
Enzyme	-	250	-	250
Incubated in water bath (37°C for 15 min)				
Na_2CO_3	1000	1000	1000	1000

Table 2: Yield of powder and crude extract of *R. mucronata* fruit

Product	Yield (%)
Fruit powder	26.11
Crude extract	11.83

R. mucronata: Rhizophora mucronata

and hypoglycemic drug and has cytotoxic activity [32]. Flavonoids can be used as antidiabetic [26] and have been introduced as hypolipidemic and antioxidant for rats that have been STZ-induced [33]. Tannins from mangrove plant have stronger antioxidant activity compared to BHT standard. Therefore, they are also potential to become antidiabetic [34].

Effect of heat treatment of extract on its inhibitory activity toward α-glucosidase

Inhibitory activity toward α-glucosidase by extract is expressed as IC_{50}, i.e. the ability of extract to inhibit 50% of enzyme activity. The lower the IC_{50} value, the higher the inhibitory activity, and vice versa. Analysis of variance (Anova) result shows that heat treatment on extract gave significant difference ($p<0.05$) on IC_{50} value. Results from *post hoc* test using Tukey can be observed in Fig. 1.

It can be seen from Fig. 1 that heating until 90°C increases the inhibitory activity of extract toward α-glucosidase, shown by lower IC_{50} value compared to IC_{50} value of unheated extract (control). This result indicates that heating until 90°C could increase the inhibitory activity related to exposure of functional group from compounds inside the extract that plays a role in inhibiting the enzyme. The highest increase in inhibitory activity toward α-glucosidase occurs in heat treatment at 70°C, which then decrease until heat treatment of 90°C. This decrease is suspected caused by the damage of functional group that plays a role in inhibiting α-glucosidase activity. The highest inhibitory activity at 70°C supported the previous research who reported that brewing temperature of 70°C on black tea gave highest inhibitory effect (96.76%) compared to brewing temperature of 100°C (91.03%) [35]. The similar result was also reported on green tea brewing [36].

Heat treatment until 100°C caused decrease in inhibitory activity of about 3.87% compared to unheated extract (Fig. 2). The slight decrease is suspected caused by tannins activity in extract. Tannins are parts of polyphenol compounds inside cell vacuoles which could act as an antioxidant. Tannins are more soluble when heated at high temperature.

Table 3: Phytochemical results of *R. mucronata* fruits powder

Analysis	Result
Alkaloids	
Mayer	+
Dragendorff	+
Flavonoids	+
Tannins	+++
Saponin	+
Triterpenoid	+
Steroid	-

+: Detected, -: Not detected. *R. mucronata: Rhizophora mucronata*

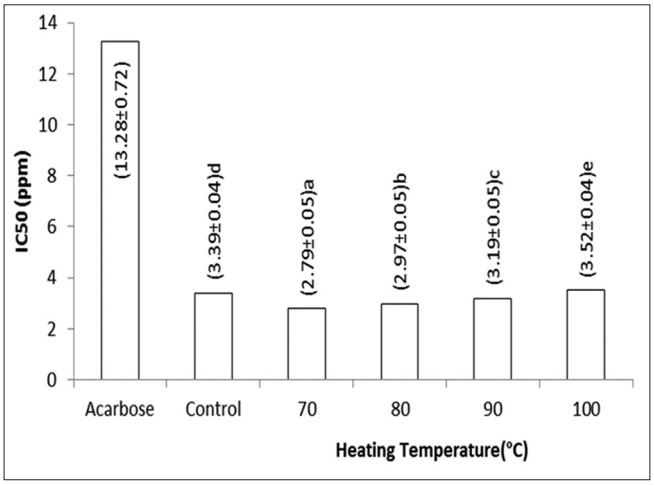

Fig. 1: Effect of heating temperature on IC_{50} value of *Rhizophora mucronata* fruit crude extract

This is supported by previous research which reported that heating at higher temperature would result in the high amount of tannins [37]. However, the quality of tannins obtained was lower because soluble tannins content was also higher.

To determine the change in inhibitory activity of extract toward α-glucosidase caused by heat treatment, percentage of change in IC_{50} value compared to control was calculated, as can be seen on Fig. 2. Positive value indicates the increase in inhibitory activity, whereas negative value indicates the decrease in inhibitory activity toward α-glucosidase. Heating of extract until 70°C increases the percentage of inhibition, compared to unheated extract, with increase percentage of 17.63%, whereas heating of extract until 100°C decreases the inhibitory activity for about (3.87%). It is because high-temperature treatment could degrade tannins compound in the extract.

R. mucronata fruit extract contains major bioactive compound, i.e., tannins, which are believed to be able to inhibit the activity of α-glucosidase. Tannins are polyphenol compounds that can precipitate protein. Enzyme is a protein that can catalyze biological reactions. Addition of tannins in enzymatic reaction of α-glucosidase could precipitate protein because protein-tannins will form complex bond and as a result, the enzyme will not be able to degrade substrate. Thus, the activity of α-glucosidase could be inhibited. Inhibition of α-glucosidase by catechins was caused by the formation of complex bond between protein and phenols, which is caused by hydrogen bond between hydroxyl group and –NH and –CO groups of protein [36].

Total phenolic content

Total phenolic content in this research was determined using Folin–Ciocalteu reagent. This method is based on reducing power of phenolic hydroxyl group. The standard used in this assay was Gallic acid. Statistical analysis result (Anova) shows that heat treatment of extract gave significant effect ($p<0.05$) on total phenolic content. Result from *post hoc* test using Tukey can be observed in Fig. 3.

Fig. 3 in general shows that total phenolic content of heated extract is higher than unheated extract (control). It is suspected caused by the change in chemical functional group or opening of phenolic functional group, as a result from heat treatment. The highest total phenolic content occurs on extracts that were heated at a temperature of 70°C and 80°C, and then decrease on extracts that were heated at temperature of 90°C and 100°C. The decrease in total phenolic compound might be because there is a phenolic compound that is sensitive to heat. One of phenolic compounds, i.e., procyanidin, is highly degraded on heating at 98°C for 90 min and 120°C for 20 min [37]. However, there are also many phenolic compounds which are heat stable and have boiling point of about 181.7°C, and even there are some phenolic compounds that can only be degraded at heating above 300°C [38]. Phenolic compounds that are oxidized will produce several products, i.e., p-benzoquinone, dicarboxylic acid and carbon dioxide.

If this result is correlated to IC_{50} value (Fig. 4), heating of extract at 70°C results in lowest IC_{50} value or highest inhibitory activity toward α-glucosidase. Thus, it can be concluded that polyphenol compounds in extract play a role in inhibiting α-glucosidase.

Total tannin content

Statistical analysis result (Anova) shows that heat treatment of extract gave significant effect ($p<0.05$) on total tannin content. The result from *post hoc* test using Tukey can be observed on Fig. 4. The phenomenon of heat treatment effect on total tannins is similar to that of total phenolic, in which heating process increases total tannin content and the highest total tannin content occurs in the extract that was heated at 70°C and even until heating temperature of 90°C. It means heating process until 90°C could open tannins groups, which makes them detected as total tannins. Heating process could denature protein that binds tannins to release the tannins [39]. Moreover, during blanching, there was denaturation of protein that caused tannins to be released from protein [40].

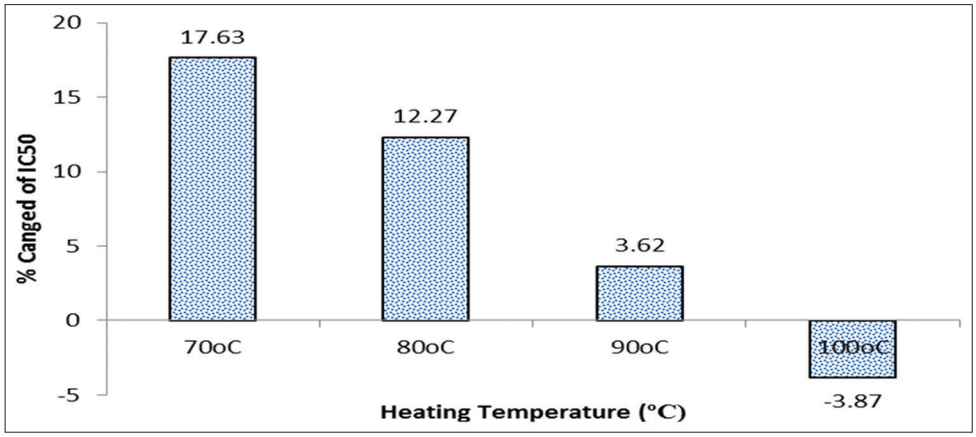

Fig. 2: The percent change of IC_{50} of *Rhizophora mucronata* extract on different heating temperature

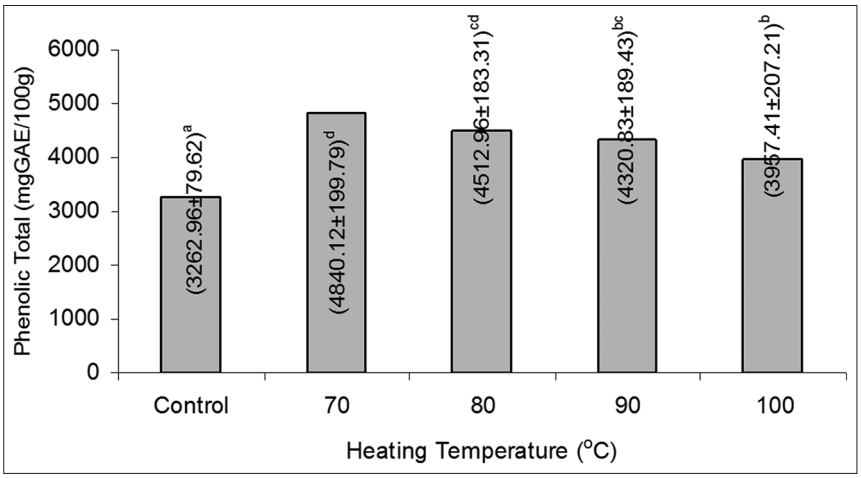

Fig. 3: Total phenolic content of *Rhizophora mucronata* crude extract on different heating temperature

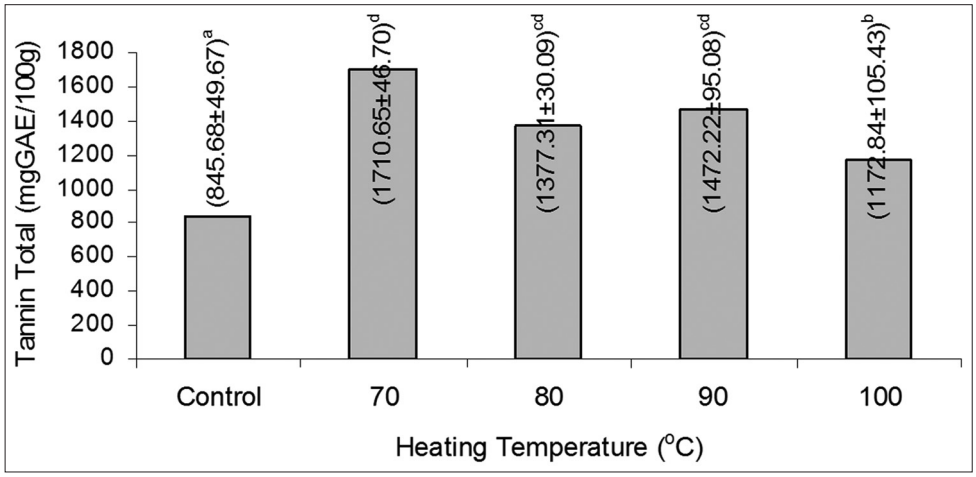

Fig. 4: Total tannins of *Rhizophora mucronata* crude extract on different heating temperature

In total tannin content, there is another compound, such as hydrocolloids [41,10]. The range of total tannins was higher than previous research, which stated that total tannins of *R. mucronata* fruit extract were about 6.20 mg/g [42]. If the result is correlated to extract activity in inhibiting α-glucosidase enzyme (Fig. 1), it shows that high total tannins (on heating at 70°C) give low IC_{50} value or high inhibitory activity toward α-glucosidase. Tannins have high ability to denature protein [43,42]. This strengthens the indication that tannins could inhibit α-glucosidase or in other words, tannins are potential to be functional antidiabetic agent.

CONCLUSIONS

Heat treatment toward *R. mucronata* crude fruit extract until 100°C could increase its inhibitory activity toward α-glucosidase with the highest inhibitory activity was with heat treatment of 70°C with IC_{50} of 2.790±0.051 ppm.

R. mucronata crude fruit extract is quite stable toward heat with heat treatment until 100°C only decreased its inhibitory activity toward α-glucosidase for about 3.67%.

Inhibitory activity of *R. mucronata* crude fruit extract toward α-glucosidase was higher compared to acarbose, makes it potential to become natural antidiabetic drugs.

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