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Research Article

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Antidiabetic and antioxidant activities of tannin extract of *Rhizophora mucronata* leaves

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ABSTRACT

Mangrove (including Rhizophora mucronata) is well known as tannin resources either condensed or hydrolyzed tannin. Condensed tannin isolated from other plants has been reported that it have activities as antidiabetic and antioxidant. Therefore, characterization condensed tannin of mature R. mucronata leaves inhibited α-glucosidase and the antioxidant activity were studied. Research method were conducted by extraction condensed tannin to get crude extract, fractination crude extract with different polarities solvent and characterization. The result showed that fractination with different polarities decreased total phenol, total tannin and total flavonoid. However, it rise total condensed tannin from 1.10 mg/100g to 2.06 mg/100g and it was followed by reduction IC_{50}α-glucosidase value from 7.65±0.50 µg/mL to 5.89 ± 0.50 µg/mL. It was lower than IC_{50} acarbose (10.60 ± 0.20 µg/mL), so it was considered condensed tannin of mature R. mucronata leaves have potency as natural drug to cure diabetic. Fractination of condensed tannin of mature R. mucronata leaves dipped IC_{50} antioxidant from 491,789.37 ± 427.59 µg/mL to 82,977.11 ± 51.15 µg/mL, but the values was higher from IC_{50} ascorbic acid (12.36 µg/mL), it can be concluded that condensed tannin of mature R. mucronata leaves did not have potency as antioxidant.

Keywords *: Rhizophora mucronata* leaves, condensed tannin, antidiabetic, antioxidant

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INTRODUCTION

Mangrove is a part of coastal plants having multifunction. Ecologically, mangrove serves as a coastal protection from abrasion and as a area overgrown by various marine organisms. Demographically, mangrove have a role as a source of timber, food, industry matter and ethno medicine. Traditionally, mangrove tannin is used for tanning and dying in leather industry. Society have utilized mangrove plant to treat diarrhea, cough, ulcer, hepatitis, stop bleeding and infection. Moreover, mangrove can be used as insecticide and pesticide [1, 2].

Utilization of the part of mangrove tree as a medicine and pesticide indicated that mangrove plants contained various active compounds or it is called bioactive. Some previous research reported that mangrove have antibacterial agents [3, 4, 5, 6, 7, 8, 9], antifungal [10, 11, 12, 13], antioxidant [14, 15, 16, 17, 18, 19], anticancer[20], and antidiabetic [21, 22, 23, 24]. According to the research result about secondary metabolite of mangrove plant reported that mangrove contained steroid, triterpene, saponin, flavonoid, alkaloid and tannin [25, 10].

Tannin almost present in the mangrove extract. In addition, tannin dominated both in*R. mucronata* leaves and *R. mucronata* fruit extracts [23,24]. Tannin content of mangrove is very high, so it was used for tanning and dying [1,10]. Consequently, the tannin was predicted that it is as main role bioactive include bioactive to cure diabetic. There is two tannin type such as condensed tannin and hydrolyzed tannin[27].There were minimum information about condensed tannin and hydrolyzed tannin, especially *R. mucronata* tannin, serving as antidiabetic. Catechin (condensed tannin) and gallic acid (hydrolyzed tannin) isolated from *Caesalpinia ferrea* rod reduced the blood

glucose level of diabetic patients [28]. This research was intended to study tannin of of mature *R. mucronata* leaves for antidiabetic.

EXPERIMENTAL SECTION

Material

Mature *R. mucronata* leaves were collected from mangrove areas Penunggul village, Pasuruan, East Java, Indonesia. Before the leaves had been extracted, it were dried and powdered. The material for extraction is acetone (SmartLab), aquadest (Hydrobatt), ascorbic acid, and hexane (SmartLab). KH_2PO4, K_2HPO4, NaOH, Bovine Serum Albumin (Calbiochem), α-glucosidase (Megazyme), Na2CO3, Substrat PNPG (Sisco Research Ltd.) were used for inhibition assay.

There were equipments used for *R. mucronata* fruit flour preparation such as plastic sheet and net. Graduated cylinder 50 mL, volumetric flask 1000 mL (Pyrex Iwaki Glass), rotary evaporator andsonicator (Branso Digital Sonifier Model 450)were used for R. mucronata fruit extraction. Futhermore, disposible cuvet 1.5mL (Brand), micropipet 10-100 µL (Socorex), micropipet 100-1000µL (Socorex), blue tip, yellow tip, white tip, test tube (Pyrex), UV-Vis (Pharo 300M) and water bath (Memmert type W 350).

Preparation of *R. mucronata*Fruit Powder

Mature *R. mucronata* leaves were sorted based on the wholeness. Then, it were air-dried until the moisture level was approximately 5%. Dried-leaves were powdered by a blender to get leaves powder, and then it kept in the refrigerator until it was used.

Extraction and Fractination of Condensed Tannin

Condensed tannin extraction was conducted based on the method [29] with modification. Mature *R. mucronata* leaves powder was measured 25 grams and 70% acetone/water (v/v) with ration 1:10 was added in the erlenmeyer. A mixture of the leaves powder and the solvent was stirred and added ascorbic acid 0.25% (w/v) to avoid oxidation. It was followed by sonication with amplitude 40 Hz for 30 minutes. The mixture was separated by a centrifuge (3000 rpm, 10 minutes) to obtain supernatant and residue. A filter paper was used to separated supernatant. Filtrat was evaporated by a rotary evaporator (60^oC). Finally, condensed tannin crude extract would be analyzed and fractionated.

Crude extract was fractionated using a separating funnel and solvent with different polarity. Firstly, crude extract was took 5 grams and it was solved with 5 mL aquadest. The solution was inserted into a separating funnel and it was added 25 mL hexane. After it had been being shaken for 15 minutes, it should be left to form 2 layers such as water layer and hexane layer. Water layer was separated and evaporated using a rotary evaporator (60°C, 10 minutes) to discard hexane. The extract was reacted with 12.5 mL choloroform in the separating funnel and it would be shaken for 15 minutes. After it had been left, it formed two layers such as water layer and chloroform layer. The chloroform layer should be discarded and the water layer remained in the separating funnel. Next, the water layer was fractionated with 12.5 mL ethyl acetate and it was shaken for 15 minutes. The solution would form two layers, namely ethyl acetate layer (top) and the water layer (bottom). The water layer was concentrated by rotary evaporator (60^oC, 5 minutes). Finally, the condensed tannin fraction of mature R. mucronata leaves obtained was stored in the freezer (4°C)

α-glucosidase Inhibitor Activity Assay

α-glucosidase inhibitor activity assay of mature *R. mucronata* leaves extract was conducted by test tube method [30] with modification. An analysis was started by preparing enzyme stock solution. Enzyme stock solution was made by solving 0.1 mL α-glucosidase in 1000 mL phosphate buffer pH 7 contained BSA. The solution was inserted intomicrotube, each microtube contained 1 mL solution. To determine dilution rate, enzyme activity assay was done on the dilution rate 10x, 20x, 30x, and 40x.

In this research, the optimum activity enzyme was obtained from a stock solotion pf enzyme in dilution 20x (Table 1).

Table 1. α-glucosidase reaction system

	Blank (B) (µL)	Control (C) (µL)	S_0 (µL)	S_1 (µL)
Sample	-	-	10	10
DMSO	10	10	-	-
Phospate buffer pH 7	490	490	490	490
PNP-G substrate	250	250	250	250
Incubated in waterbath (37°C for 5 minutes)				
Phospate buffer pH 7	250	-	250	-
Enzyme α-glucosidase	-	250	-	250
Incubated in waterbath (37°C for 5 minutes)				
Na_2CO_3	1000	1000	1000	1000
Absorbance 400 nm				

The mixture consisted of 10 µL samplesolved in DMSO, 490 µL phospate buffer (pH 7), 250 µL PNP-G substrate 20 mM homogenated by vortex, and it was incubated (37°C for 5 minutes). Then, the mixture was added 250 µL α-glucosidase (C and S_1) and 250 µL phospate buffer pH 7 (B and S_0), and it was incubated (37°C for 5 minutes). The reaction was stopped by adding 1000 µL sodiumcarbonate 200 mM.

% Inhibition was calculated with the formula [(C-S) / C] x 100%; C = control absorbance (DMSO) without sample (C-B); S = sample absorbance (S_1-S_0)

Inhibition enzyme activity presented in IC_{50} was calculated using linear regression formula, sample concentration as X-axis and % inhibition as Y-axis, in order that it was formulated Y= a + bX. IC_{50} value was calculated by formula (50–a)/b.

Total Phenolic Content

The total phenolics was measured by spectrophotometry and it was conducted based on Folin-Ciocalteu reaction method [31]. 0.5 mL sample extract was reacted with Folin-Ciocalteu (1:10), and it was homogenized by a vortex. The solution was left in the dark room for 4 minutes and then it added with 2 mL sodium carbonate 7.5% (w/v). The mixture was homogenized and it was incubated in the dark room for 2 hours. The absorbance of the solution was measured with spectrophotometer wavelength of 760 nm. Total phenolic was presented as milligram gallic acid equivalent (mg GAE/100g dry weight) sample.

The standard solution is gallic acid solution with concentration 50, 100, 150, 200, 250 ppm. The result of gallic acid with various concentrations was plotted in the chart with concentration gallic acid concentration as horizontal axis (X) and absorbance as vertical axis (Y), to obtain the linear equation.

Total Flavonoid

The total flavonoid of the mature *R. mucronata* leaves extract was assayed based on the method described by [32]. 0.25 mL sample extract was reacted with 1.25 mL distillated water and 75 µL $NaN0_2$5%, and it was left for 6 minutes. 150 µL $AlCl_3$2% was added into the mixture and it was left for 5 minutes. 0.5 mL NaOH 1 M and 775 µL distillated waterwere added into the mixture and it was left again for 5 minutes. 0.5 mL NaOH and 775 µL distillated water were added into the mixture. Absorbance was measured with spectrophotometer at a wavelength of 415 nm. The total flavonoid of mature *R. mucronata* leaves extract was presented in mg QE/100 gram dry weight sample. Quercetin solution was used by 50, 100, 150, 200, 250, 300, 350 ppm. The result of various concentration analysis was plotted into a graph with quercetin concentration as the X-axis and absorbance as the Y-axis.

Condensed Tannin Analysis

Total tannin was determined [33] according to oxidative depolymerization condensed tannin reaction with butanol-HCl method. 0.5 mL extract was inserted into Hungate tubes. It was added with 3 mL butanol-HCl reagents (95:6 v/v) and 0.1 mL ferric reagent, and then it was homogenized by vortex. The Hungate tube was closed and inserted into water bath for 60 minutes (97-100°C). The tubes contained solution was chilled, after that the absorbance was measured with spectrophotometer at a wavelength of 550 nm. Blank was measured. Blank is the mixture without heating. Condensed tannin was calculated with the formula = (Absorbance a wavelength of 550 nm x 78.26 x Diluted Factor) / % Dry Matter.

Antioxidant Activity DPPH METHOD

Antioxidant activity of the mature *R. mucronata* leaves extract was assessed according to the radical scavenging using DPPH method (1,1-Diphenyl-2-2picrylhydrazyl)[32]. 1.0 mL DPPH solution 0.4 mM and 0.8 mL methanol extract sample were reacted. Control was prepared by adding 1 mL DPPH solution 0.4 mM with 0.8 mL methanol.

Blank was used 1.8 mL methanol. The inhibition (%) of free radical was calculated by the formula = [(Control absorbance – sample absorbance) / Control absorbance] x 100%.

Antioxidant activity presented by IC_{50} was expressed using a linear regresion equation, sample concentration as X-axis and % inhibition as Y-axis to get Y= a + bX. IC_{50} value was calculated using formula (50-a)/b.

RESULTS AND DISCUSSION

Chemical Composition and Inhibitory Activity of Mature *R. mucronata* Leaves Extract

Table 2. Chemical composition and inhibitory activity of mature *R. mucronata* leaves extract

Parameter	Crude Extract Condensed Tannin	Fraction Condensed Tannin
Total Phenolic (mg GAE/100g)	189,759.69 ± 13,389.94	9,378.30 ± 223.47
Total Tannin (mg/100g)	110,000.00 ± 11,422.01	248.06 ± 35.52
Total Flavonoid (mg/100g)	7,291.23 ± 315.37	87.83 ± 11.62
Total condensed tannin (mg/100g)	1.10 ± 0.00	2.06 ± 0.00
α-glucosidase [IC_{50} (µg/mL)]	7.65±0.50	5.89 ± 0.50
Antioxidant activity (IC_{50} µg/mL)	491,789.37 ± 427.59	82,977.11 ± 51.15
Inhibition α-glucosidase Acarbose (IC_{50}µg/mL)	10.60± 0.20	

As shown in Table 2, $IC_{50}$of condensed tannin of *R. mucronata* leaves crude extract was higher than $IC_{50}$of condensed tannin of *R. mucronata* leaves fraction. The inhibitory activity of condensed tannin crude extract towards α-glucosidase was lower than the condensed tannin fraction. It was showed that 1) separation or reduction other compounds of the crude extract enhanced condensed tannin activity to inhibit α-glucosidase; 2) less synergy among condensed tannin and other compounds inhibited α-glucosidase activity.

Furthermore, the increase of condensed tannin fraction activity in order to inhibit α-glucosidase was followed by reducing total phenolics, total tannin, total flavonoid but rising condensed tannin level. Condensed tannin such as epicatechin-(4β,8)-epicatechin gallate (B2-3′-O-gallate), epicatechin gallate (ECG) dan 2-(4-hydroxyphenyl) ethyl 3,4,5-trihydroxybenzoate (HETB) isolated from*Rhodia crenulata*have been proved as α-glucosidase inhibitor [34].

The potency of phytochemical compounds such as tannin and flavonoid against α-glucosidase activity was reported [35,36]. Condensed tannin has role as antidiabetic by inhibiting α-glucosidase[35]. In addition, tannin delay glucose absorbtion in the human intestine [37]. Flavonoid showed the strongest inhibitory effect and more specific towards α-glucosidase[36]. α-glucosidase inhibitory mechanism was expected by hydroxylation binding and ring β substitution. It would delay carbohydrate hydrolisis, glucose absorbtion and inhibit the breakdown of carbohydrate into glucose [38].

$IC_{50}$of condensed tannin of *R. mucronata* leaves crude fraction reached 5.89 ± 0.50 µg/mL, and IC_{50} acarbose was 10.60 ± 0.20 µg/mL. As shown in Table 2, inhibitory activities of crude extract and tannin fraction towards α-glucosidase activity can be compared with the standard drug for diabetic such as acarbose. It can be seen that the potency of condensed tannin extracted from mature *R. mucronata* leaves have higher towards α-glucosidase activity than acarbose. Consequently, condensed tannin isolated from mature *R. mucronata* leaves can be considered as drug to cure diabetic patient. Moreover, the mechanism tannin as antidiabetic was the inhibition gastrointestinal enzyme and glucose transporter in the intestine [28].

Based on the antioxidant activity, fractination increased antioxidant activity. It was shown by IC_{50} values. IC_{50} condensed tannin *R. mucronata* leaves fraction was lower than IC_{50} condensed tannin *R. mucronata* leaves crude extract. It indicated that condensed tannin *R. mucronata* leaves have potency as antioxidant. However, IC_{50}ascorbic acid12.36 µg/mL[39,40] was lower compared to the IC_{50} condensed tannin *R. mucronata* leaves fraction (82,977.11 ± 51.15 µg/mL). It explained that condensed tannin *R. mucronata* leaves fraction have less potency as antioxidant.

CONCLUSION

Fractination of condensed tannin *R. mucronata* leaves crude extract with different polarity solvent reduced total phenolic, total tannin, total flavonoid but it increase condensed tannin content.

The rise of condensed tannin declined IC_{50} α-glucosidase value or increase inhibition activity of condensed tannin of *R. mucronata* leaves against α-glucosidase.

IC_{50} condensed tannin *R. mucronata* leaves fraction was lower than IC_{50} standard drug acarbose, it means that condensed tannin *R. mucronata* leaves fraction have potency as natural product for antidiabetic.

The activity of condensed tannin fraction of *R. mucronata* leaves were lower than ascorbic acid, and less potential as antioxodant.

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