RESEARCH ARTICLE

Free Radical Scavenging and α-/β-glucosidase Inhibitory Activities of Rambutan (*Nephelium lappaceum* L.) Peel Extract

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Received date: Mar 6, 2015; Revised date: Apr 6, 2015; Accepted date: May 25, 2015

Abstract

BACKGROUND: Diabetes mellitus (DM) is associated with oxidative reaction and hyperglycemic condition. Human body has an antioxidant defense system toward free radical, but overproduction of free radical causing imbalance condition between the free radical and the antioxidant defense in the body that lead to several diseases, including DM. Glucosidase is an enzyme that hydrolize carbohydrates causing increase of blood glucose level, so by inhibiting this enzyme blood glucose level in plasma could be effectively decreased. Rambutan (*Nephelium lappaceum* L.) peel has been reported to have many potential roles, such as antioxidant and anti-glycemia. Therefore our current study was conducted to evaluate possible effectivity of Rambutan peel to scavenge free radical and to inhibit α- and β-glucosidases.

METHODS: Rambutan peel extraction (RPE) was performed based on maceration method. Geraniin was used as control. For antioxidant study, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging test was performed. For glucosidase inhibitory activity study, α- and β-glucosidases inhibitory activity tests were performed. Results were analyzed for median of Inhibitory Concentration (IC_{50}).

RESULTS: The scavenging activity of RPE was comparable with Geraniin. Meanwhile, the α-glucosidase inhibitory activity of RPE was higher than the one of Geraniin. The α-glucosidase-inhibitory-activity IC_{50} of RPE and Geraniin were 0.106±0.080 µg/ml and 16.12±0.29 µg/ml, respectively. The β-glucosidase inhibitory activity of RPE was also higher than the one of Geraniin. The β-glucosidase-inhibitory-activity IC_{50} of RPE and Geraniin were 7.02±0.99 µg/ml and 19.81±0.66 µg/ml, respectively. .

CONCLUSION: Since RPE showed comparable free radical scavenging activity with Geraniin and higher α- and β-glucosidases inhibitory activities than Geraniin,, RPE could be suggested as a promising antioxidant and anti-glycemic agent.

KEYWORDS: *Nephelium lappaceum* L., rambutan, hypoglycemic, antioxidant, free radical, diabetes mellitus, glucosidase, DPPH

Indones Biomed J. 2015; 7(3): 157-62

Introduction

Diabetes mellitus (DM) is among the largest contributors to global mortality through its long term complications.(1) Free radicals act significant role in development of DM. Insulin resistance and β-cell dysfunction are caused by oxidative stress.(20) Antioxidants can interfere the oxidation process by reacting with free radical, chelating catalytic metals, and also by acting as oxygen scavenger.(3) A free radical is a

single unpaired electron. Reactive oxygen species (ROS) is one of the most concern free radical. The human body has an antioxidant defense system toward free radical, but overproduction of free radical causing imbalance condition between the free radical and the antioxidant defense in the body that lead to several diseases.(4-7) Free radical scavenger properties are needed in DM treatment.

DM is a common disease which can be characterized by hyperglycemic condition or abnormally high plasma glucose level.(8) Control of postprandial blood glucose level is critical in treating DM.(9) Glucosidase is an enzyme that hydrolyze carbohydrates causing increase of blood glucose level, so by inhibiting this enzyme blood glucose level in plasma could be effectively decreased.(10) One of therapeutic approaches to treat DM is to retard the absorption of glucose via inhibitions of several glucosidase including α- and β-glucosidases. Elevation of blood sugar following a carbohydrate meal can be decreased by inhibiting this enzyme.(11,12).

The plant has been suggested as a rich source for anti-diabetic drug.(13) Rambutan (*Nephelium lappaceum* L.) is a tropical fruit from Southeast Asia. This fruit was shown to exhibit high antioxidant activity.(14) Therefore our current study was conducted to evaluate possible effectivity of Rambutan peel to scavenge free radical and to inhibit α- and β-glucosidases.

Methods

Rambutan Extraction

Extraction was performed based on maceration method. (15-19) Rambutans were collected from Kesamben-Blitar plantation, East Java, Indonesia. Dried and milled rambutan peels were soaked in 70% distillated ethanol, then were evaporated. Geraniin, a typically ellagitannin isolated from *Geranium thunbergii*, was used as control due to its potential as glucose inhibitor and free radical scavenger. Geraniin was commercially available (Cat. No. 60976-49-0, Cengdu Biopurify Phytochemicals, Chenngdu, China).

2,2-diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Test

Fifty µl sample/extract was introduced in 96-well microplate and 200 µl of 0.077 mmol DPPH in dimethyl sulfoxide (DMSO) were added. The mixture was shaken vigorously and incubated in a dark room, at room temperature, for 30 min. Afterthat, measurement at 517 nm absorbance using a microplate reader (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific, Waltham, MA, USA) was performed. For negative controls, 250 µl DPPH was used. For blank, 250 µl methanol was used.(18-22) . The DPPH scavenging activity (%) was calculated as follows:

Scavenging Activity (%)=(Ac-As)/Ac×100

As: sample absorbance

Ac: negative control absorbance (without sample)

α-glucosidase Inhibitory Activity Test

The α-glucosidase inhibitory activity was tested with modification.(23,24) Briefly, each sample was diluted in 10% DMSO. Five µL of sample, 25 µl of 200 mM p-nitrophenyl-a-glucopyranoside, 45 µl phosphate buffer saline (PBS) (pH.7), 25 µl of *Saccharomyces sp*. yeast α-glucosidase were introduced in the microplate and incubated at 37°C for 5 min. The reaction was stopped by adding 100 µL of 200 mM Na_2CO_3 and then measured at 400 nm using a microplate reader. For control, 10% DMSO merely was used. The α-glucosidase inhibitory activity was calculated as follows:

α-glucosidase inhibitory activity (unit/L)=(Ac-As)/Ac ×100

As: sample absorbance

Ac: negative control absorbance (without sample)

β-glucosidase Inhibitory Activity Test

The β-glucosidase inhibitory activity was assayed according to Sigma-Aldrich protocol. Twenty µl of each sample was transferred into 96 well plate. Then 200 µl master mix reaction was added. Initial absorbance was measured at 405. Then the samples were incubated at 37°C for 20 min. then the final absorbance was measured at 405 mn. The ß-glucosidase inhibitory activity was calculated as follows:

β-glucosidase inhibitory activity (unit/L)=(Af-Ai)/(Ar-Aw) ×250

Af: final absorbance

Ai: initial absorbance

Ar: calibrator absorbance

Aw: water absorbance

Results

With the maseration method, from 400 g of dried and milled rambutan peel, we obtained 45 g of extract. Rambutan peel extract (RPE) was then tested for the DPPH scavenging activity, α- and β-glucosidases inhibitory activities.

DPPH Free Radical Scavenging Activity

DPPH free radical scavenging activity can be used to determine antioxidant capacity of plant. The scavenging activities of RPE and Geraniin can be seen at Figure 1, while the IC_{50} values were shown in Table 1. The scavenging activity of RPE was comparable with Geraniin.

α-glucosidase Inhibitory Activity

The α-glucosidase inhibitory activities of RPE and Geraniin were shown in Table 3. The assay was measured in triplicate for each sample. The α-glucosidase inhibitory activity of RPE was higher than the one of Geraniin. The α-glucosidase-inhibitory-activity IC_{50} of RPE was 0.106±0.080, while the one of Geraniin was 16.12±0.29.

β-glucosidase Inhibitory Activity

The β-glucosidase inhibitory activity is determined by a reaction in which β-glucosidase hidrolizes p-nitrophenyl-b-D-glucopyranoside resulting in the formation of a colorimetric product at 405 nm.(21) The result of this test is presented in Figure 2 and Table 2. The β-glucosidase inhibitory activity of RPE was higher than the one of Geraniin. The β-glucosidase-inhibitory-activity IC_{50} of RPE was 7.02±0.99, while the of Geraniin was 19.81±0.66.

Table 1. DPPH Free Radical Scavenging Activity IC_{50} of RPE and Geraniin.

Samples	Equation	R^2	IC_{50}	Average IC_{50}
RPE Test 1	y=13.935x+10.346	0.959	2.85	
RPE Test 2	y=13.831x+9.8118	0.9633	2.91	
RPE Test 3	y=14.212x+8.1402	0.9644	2.95	
Average of RPE	y=13.933x+9.4326	0.9634	2.91	2.90±0.05
Geraniin Test 1	y=13.505x+11.317	0.9247	2.86	
Geraniin Test 2	y=13.629x+11.141	0.908	2.85	
Geraniin Test 3	y=13.679x+10.655	0.9225	2.88	
Average of Geraniin	y=13.634x+11.038	0.9203	2.86	2.86±0.01

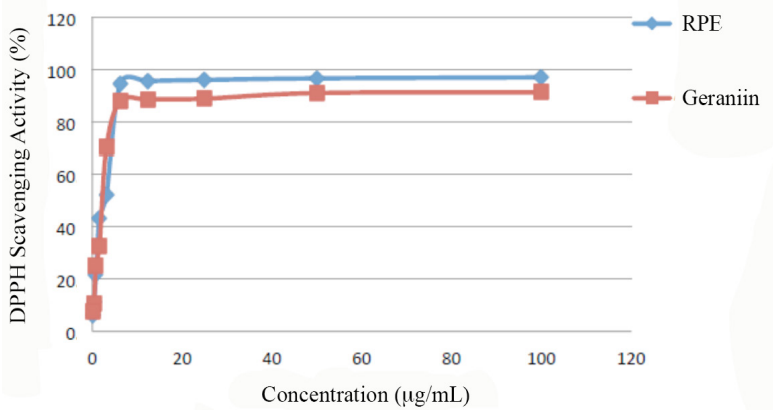

Figure 1. DPPH Free Radical Scavenging Activity of RPE and Geraniin. RPE and Geraniin were diluted in methanol to reach the final concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781, 0.391, 0.195 µg/mL.

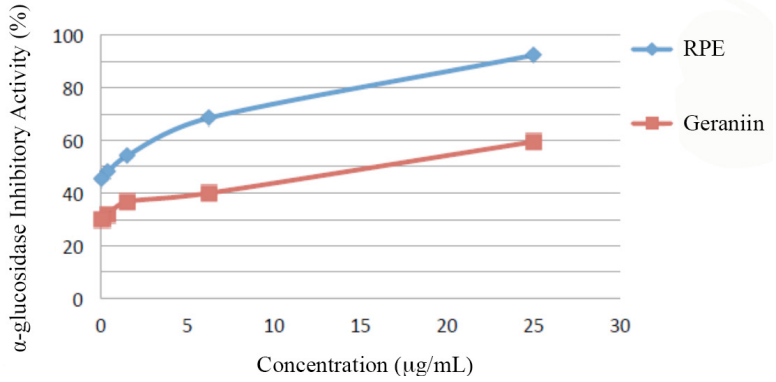

Figure 2. The α-glucosidase inhibitor activity of RPE and Geraniin. RPE and Geraniin were diluted in 10% DMSO to reach the final concentrations of 25, 6.25, 1.563, 0.398, 0.078 µg/mL.

Table 2. The α-glucosidase inhibitor activity IC_{50} of RPE and Geraniin.

Samples	Equation	R^2	IC_{50}	Average IC_{50}
RPE Test 1	y=1.8298x+49.974	0.9353	0.014	
RPE Test 2	y=1.7519x+49.748	0.9303	0.144	
RPE Test 3	y=1.7494x+49.718	0.9277	0.161	
Average of RPE	y=1.7882x+49.993	0.9299	0.004	0.106±0.080
Geraniin Test 1	y=1.0758x+32.343	0.9566	16.41	
Geraniin Test 2	y=1.1158x+32.324	0.9793	15.84	
Geraniin Test 3	y=1.1348x+31.734	0.9672	16.1	
Average of Geraniin	y=1.1088x+32.134	0.9711	16.11	16.12±0.29

Table 3. The β-glucosidase inhibitory activity IC_{50} of RPE and Geraniin.

Samples	Equation	R^2	IC_{50}	Average IC_{50}
RPE Test 1	y=1.2443x+41.75	0.9276	6.63	
RPE Test 2	y=1.0288x+43.532	0.9378	6.29	
RPE Test 3	y=1.0799x+41.21	0.8905	8.14	
Average of RPE	y=1.1176x+42.164	0.9274	7.01	7.02±0.99
Geraniin Test 1	y=0.945x+30.709	0.9132	20.41	
Geraniin Test 2	y=1.0304x+30.321	0.8539	19.1	
Geraniin Test 3	y=0.9038x+31.632	0.7313	19.92	
Average of Geraniin	y=0.9597x+30.887	0.8552	19.92	19.81±0.66

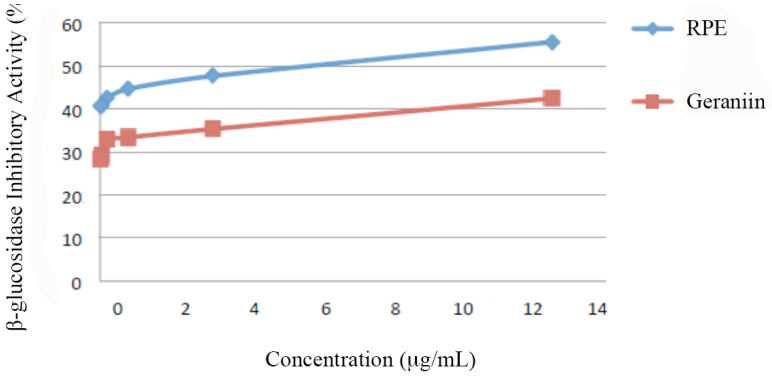

Figure 3. The β-glucosidase inhibitor activity of RPE and Geraniin. RPE and Geraniin were diluted in 10% DMSO to reach the final concentrations of 25, 6.25, 1.563, 0.398, 0.078 µg/mL.

Discussion

The peel of rambutan, which is usually discarded, was found to have extremely high antioxidant activity.(25) Antioxidant can stabilize free radicals that could cause oxidative damage to the cells. Our result shows that RPE and Geraniin have comparable antioxidant activities. This result confirmed previous study reporting that RPE has the highest free radical scavenging activity in comparison to mangosteen and langsat peels.(26) RPE has antioxidant activity due to its phenolic component.(27) Geraniin was reported to be the major compound of RPE.(28) Previous study reported the Geraniin free radical scavenging activity by using the radical galvinoxil (IC_{50} = 1.9 µM) and 3-ethylbenzthiazoline-6-sulfonate (ABTS) (IC_{50} = 6.9 µM), and indicated that Geraniin has similar antioxidant activity with RPE.(29) The high potential for scavenging free radical could inhibit spreading of oxidation.(30) RPE which showed high antioxidant activity through free radical scavenging activity, similar to Geraniin, could be potential for DM patients.

Glucosidase inhibitors play a role for disruption of the activity of glucosidase, an enzyme that cleaves the glycosidic bond. These inhibitors have played a vital role in the functions of glucosidases in living system by modifying or blocking specific metabolic processes. This led to several applications of these chemical entities in agriculture and medicine.(31) The α- and β-glucosidases are carbohydrate hydrolyzing enzymes that related to metabolic disorder such as DM. Inhibition carbohydrate hydrolyzing enzymes could be therapeutic approach to decrease hyperglycemia.(32,33) Our present study shows that both RPE has activities to inhibit α- and β-glucosidases. Previous study state that Geraniin and RPE can be potential sources as anti-glycemic agents.(34)

The α-glucosidase inhibitors seem to be the most effective in reducing hyperglycemia that occured in DM by

delaying the absorption of carbohydrate in small intestine. Importantly, these agents could reduce the blood glucose without increasing insulin secretion and do not cause hypoglycemia or weight gain. In individual with type 2 DM, the inhibition of α-glucosidase activity can reduces hemoglobin A1c (HbA1C) and postprandial insulin levels. In addition, treatment with α-glucosidase inhibitor can improve lipid metabolism, reduce fasting plasma glucose levels, and improve insulin sensitivity.(34)

Conclusion

RPE has the property of free radical scavenging and α- and β-glucosidases inhibitory activities. The present study shows that RPE and Geraniin have a potency as antioxidant and anti-glycemic agents.

Acknowledgement

We gratefully acknowledge the full support from Biomolecular and Biomedical Research Center, Aretha Medika Utama.

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