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α-Glucosidase Inhibitory Activity and Hypoglycemic Effect of *Phaleria macrocarpa* Fruit Pericarp Extracts by Oral Administration to Rats

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Abstract: α-Glucosidase *in vitro* inhibitory activity and hypoglycemic effect by oral administration in rats of *Phaleria macrocarpa* fruit extracts have been conducted. The hypoglycemic activity of α-glucosidase inhibitor is resulted from a competitive, reversible inhibition of pancreatic α-amylase and membrane bound intestinal hydrolase enzyme such as isomaltase, maltase and sucrase. In diabetic patients, inhibition of these enzymes results delayed glucose absorption and a lowering a postprandial hyperglycemia. The purpose of this study is to investigate the hypoglycemic activity of fruit extracts of *Phaleria macrocarpa* (Scheff) Boerl. as an α-glucosidase inhibitors by *in vitro* and *in vivo* experiments. α-Glucosidase inhibitory activity evaluation showed that the *n*-butanol of young and ripe fruit extracts have the highest activity, followed by ethyl-acetate and methanol extracts. By oral administration to rats, the hypoglycemic activity has been showed that the boiled water extract of ripe fruit with dose of 6.20×10^{-3} and 1.24×10^{-3} mg g^{-1}, *n*-butanol extract of ripe fruit with dose of 1.81×10^{-3} and 3.62×10^{-3} mg g^{-1}, respectively, decreased the blood glucose concentration of rats after treated with 80% w/v sugar solution, being comparable to those of acarbose 1.00×10^{-3} mg g^{-1} rat as the positive control.

Key words: *Phaleria macrocarpa*, fruits, α-glucosidase inhibitor, hypoglycemic, activity, *n*-butanol extract

INTRODUCTION

α-Glucosidase inhibitor has been used to treat type 2 diabetes mellitus. This drug does not increase insulin secretion. The antihyperlipidemic activity of α-glucosidase inhibitor comes from reversible inhibition on hydrolase, α-amylase pancreatic enzymes and intestine digestive enzymes, such as, isomaltase, sucrase and maltase. These enzymes hydrolyze food carbohydrates to glucose and other monosaccharide. The α-glucosidase inhibitor inhibits the glucose absorption in the intestine acting as antihyperglycemia after carbohydrate intake (Slagle, 2002; Bayer, 2004). Selected plant extracts, such as, *Phaleria macrocarpa* fruit pericarp, avocado (*Persea americana* L.) seed, *salam* (*Syzygium polyantha* Wight) leaves, *jamblang* (*Syzygium cumini* Merr.) bark, *sukun* (*Arthocarpus communis* Forst) leaves, *kesumba* (*Canarium secundum* Benn.), *cocor bebek* (*Kalanchoe pinnata* (Lamk) Pers) and *bungur* (*Lagerstroemia flos reginae* Retz.) leaves, showed significant α-glucosidase inhibitory activity in our laboratory experiments.

Phaleria macrocarpa (Scheff.) Boerl. {*syn. P. papuana* Warb. var. Wichanii (Val.) Back. (Thymelaeaceae), its local names *Simalakama* (Sumatra), *Makutodewo* (Java)}, is an endemic Papua plant used traditionally to treat various diseases, such as, diabetes, cancer, cardiovascular, rheumatic, high blood pressure, acne and insect bites. Qualitatively this plant showed the presence of alkaloid, lignan, saponin, terpenoid and poly-phenol. In the latex which showed in secticide activity, contained toluquinone, ethylquinone, octanoic acid, 1-nonene, 1-undecene, 1-pentadecene, 1-heptadene and 6-alkyl-1-4-naphtoquinone (Kardono, 2003). From fruit pericarp, a known cytotoxic lignan 5-[4 (4-methoxy-phenyl-tetrahydrofuro- [3,4-c]furan-1-yl] -benzene 1,2,3-triol, has been isolated (Lisdawati, 2002).

The present study reports the evaluation of α-glucosidase inhibitory activity (*in vitro*) and hypoglycemic effect of *Phaleria macrocarpa* fruit pericarp extracts by oral administration to rats.

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MATERIALS AND METHODS

Plant material: Young (green color) and old-un ripe (red color) fruits were collected from Research Center for medicinal plants and spices, Bogor, Indonesia. The pericarp of the fruits was sliced, sun-dried and powdered. Voucher plant specimen was deposited at Herbarium Bogoriense, Bogor, Indonesia.

Extraction procedures: About 200 g of powdered fruit pericarp were dissolved in MeOH 1.5 L (thrice), filtered and evaporated (rotary evaporator 40°C) to get crude MeOH extract (85 g for mature and 98 g for young fruits). The 50 g MeOH extracts then were dissolved in 2l ethyl acetate-water (1:1) to get ethyl-acetate-soluble part and *n*-butanol-soluble part. To the *n*-butanol-soluble part, 1 L of water was added. The ethyl-acetate, *n*-butanol and water part were evaporated (rotary evaporator 40°C) to get ethyl-acetate, *n*-butanol, water extracts, 5, 25, 20 g (for young fruits) and 8, 25 and 17 g (for mature fruits) respectively. Boiled water extract was made by boiled 20 g of the fruits with 300 mL water, as in traditional application. The boiled water extract was filtered and evaporated (rotary evaporator 40°C) to get 5 g (for young fruit) and 4 g (for old unripe fruit), respectively. All extracts were screen on using standard analysis procedure for their phytochemical constituents, especially for their alkaloid, flavonoid, phenol, saponin, tannin, steroid-triterpenoid, carbohydrate, protein and amino acid contents (Harborne, 1973).

Animals: Male rats strain Wistar having normal activity about 6 months old weight 250-350 g each, were selected for the experimental study.

α-Glucosidase inhibitory activity evaluation: α-Glucosidase inhibitory activity evaluation of the extracts was performed using established procedure (Lee and Lee, 2001; Prashanth *et al*., 2001; Kardono, 2002). α-Glucosidase enzyme solution was dissolved in phosphate-buffer solution (pH 7) containing 200 mg albumin serum. Before its application, 1 mL of the enzyme solution was diluted 25 times with the buffer solution. The reaction mixture consisting of 250 μL of 20 mM *p*-nitrophenyl α-D-glucopyranose as the substrate, 490 μL of 100 mM phosphate buffer (pH 7) and 10 μL of the extract dissolved in DMSO was prepared. The reaction mixture then was water-bath incubated at 37°C for 5 min. The enzyme solution (250 μL) was added, and keep the solution incubated for 15 min. The enzyme reaction was stopped by addition of 100 μL, 200 mM sodium carbonate solution. The resulted *p*-nitrophenol from the reaction was measured at λ 400 nm. As positive control, the reaction of 1% of quercetin solution was measured. The commercial α-glucosidase anti-diabetic drug, glucobay was available in the laboratory only in a form of sustain release tablet, therefore, quercetin is selected for positive control for *in vitro* evaluation. Extract concentrations for activity evaluation were 6.25, 12.5, 25, and 50 ppm and 6.25, 12.5, and 25 ppm (μg mL^{-1}) for quercetin.

Hypoglycemic evaluation using oral glucose tolerance test on rats: Before the application, all experimental rats were adapted for 2 weeks.

Determination of extract doses and the positive control acarbose (Glucobay): Glucobay (containing acarbose), was selected as the positive control. Extract dose was determined followed the traditional application. About 5-6 dried sliced of fruit pericarp (about 300 mg, was boiled with 5 glasses of water for few minutes up to the water become 3 glasses. The water was drink, one glass per dose (Winarto, 2003). For acarbose, the dose was calculated as for 50 kg for application in diabetic patient. Depend on the average weight of rats, the application to experimental rats for positive control and the extracts can be calculated.

Determination of sucrose solution dose for oral application to rats: In this experiment 9 healthy rats were divided into 3 groups, group I, II and III. Each group of rats was taken for fasting for 18 h. After fasting, the blood glucose was determined. Group 1, 2 and 3 were given orally 1 mL each of 40, 60, and 80% w/v. Then, the blood glucose was evaluated after 30, 60, 120 and 180 min.

Evaluation of hypoglycemic activity: In this experiment 15 healthy rats were divided into 3 groups, group A, B and C.

Group A: The rats were given orally the commercial tablet, glucobay (acarbose), dose 1.00 x 10^{-3} mg w/w of rat).

Group B: The rats were given orally the boiled water extract 1× conversion of dose (6.20×10^{-4} mg g^{-1} w/w of rat) and 2 × conversion of dose (1.24×10^{-3} mg g^{-1} w/w of rat).

Group C: The rats were given orally the *n*-butanol extract 1× conversion of dose (1.81×10^{-3} mg g^{-1} w/w of rat) and 2× conversion of dose (3.62×10^{-3} mg g^{-1} w/w of rat).

Rats in each group were fasting for 18 h. The blood glucose was determined. Then, rats in group A were given

acarbose tablet orally; rats in group B were given the boiled water extract 1× conversion of dose and rats were given orally the *n*-butanol extract 1 × conversion of dose. Five minutes later, to each rat in each group, one mL of 80% w/v sucrose solution was given orally. The blood glucose of each rat was determined after 30, 60, 120 and 180 min. The same manner was conducted for boiled water extract and *n*-butanol extract for 2 × conversion of dose.

For negative control (group A1, B1 and C1) one rat taken from each group was un-treated, neither acarbose nor the extract. The rats were taken for fasting for 18 h, then was given orally 1 mL each sucrose solution 80% w/v. Then the blood glucose was evaluated after 30, 60, 120 and 180 min.

Evaluation of glucose concentration in blood rats using glucose test strip: From each rat, the blood was taken from 70% v/v alcohol cleaned tail, and the blood was dropped on glucose test strip. The blood glucose was read from digital glucose meter (Anonymons, 2004).

Data analysis: The data of the experiment results were analyzed statistically using Complete Randomized Design. When there was a significant difference, the analysis was continued with Duncan test, at $\alpha = 0.05$ (Mendenhall and Sincich, 1992).

RESULTS AND DISCUSSION

P. macrocarpa fruits consist of pericarp and seeds. The pericarp is known as medicinal for various diseases treatment and the seeds are toxic. The yield of methanol extract of young and old-un ripe pericarp fruits was 42 and 49%, respectively. From young fruits methanol extract, the ethyl acetate-, *n*-butanol- and water-soluble parts were 8, 50, and 37% and from the old fruits methanol extract, the ethyl acetate-, and *n*-butanol- and water- soluble part was 15, 48 and 34%, respectively.

The α-glucosidase inhibition test is performed to methanol, ethyl acetate, *n*-butanol and boiled water extracts of *P.macrocarpa* young and old fruits pericarp at concentration 6.25, 12.5, 25, and 50 ppm, respectively. At concentration of 50 ppm, the *n*-butanol and ethyl-acetate part-extracts of *P. macrocarpa* young fruit pericarp showed the highest inhibitory activity, 71.11 and 70.93%. Methanol and boiled water extracts showed α-glucosidase inhibitory activity of 40.44 and 26.53% (Table 1).

At concentration of 50 ppm (μg mL^{-1}), the *n*-butanol, ethyl-acetate, methanol and boiled water extracts of *P.macrocarpa* old fruit pericarp showed the α-glucosidase inhibitory activity, in turn 69.90, 42.27, 37.09 and 33.01%, respectively (Table 2). The water part extract of both old and young fruits pericarp did not show any α-glucosidase inhibitory activity.

The traditional application use only for old fruits pericarp, the hypoglycemic evaluation using rats were using *n*-butanol part and boiled water extracts of old fruits pericarp.

At the first step of the experiment is performed the oral glucose tolerance test for determining the sucrose solution dose for oral application to rats (Table 3). The data of the test showed that the highest blood-glucose concentration of group 1 was reached after 1 h and decreasing to normal after 3 h. The result of the oral glucose tolerance test of group 3 is the highest compared to group 1 and 2. This data showed that 80% sugar intake having ability to increase the blood-glucose concentration acceptable to rats. Therefore, for further

Table 1: α-Glucosidase inhibitory activity of methanol, ethyl acetate, *n*-butanol and boiled water extracts of *P. macrocarpa* young fruit pericarps

						Standard deviation				
Concentration (ppm)	Methanol	Ethyl acetate	n-buthanol	Boiled water	Quercetin	Methanol	Ethyl acetate	n-buthanol	Boiled water	Quercetin
6.25	3.82	14.02	34.14	0	14.05	11.053	2.362	7.184	4.522	0.266
12.5	11.47	30.72	54.75	0	26.22	11.100	3.638	4.166	4.416	1.458
25	20.75	45.15	63.64	11.45	51.35	12.954	3.990	3.524	3.145	3.385
50	40.44	70.93	71.11	26.53		11.560	1.517	5.493	1.617	

Table 2: α-Glucosidase inhibitory activity of methanol, ethyl acetate, *n*-butanol and boiled water extracts of *P. macrocarpa* old fruit pericarps

						Standard deviation				
Concentration (ppm)	Methanol	Ethyl acetate	n-buthanol	Boiled water	Quercetin	Methanol	Ethyl acetate	n-buthanol	Boiled water	Quercetin
6.25	0	8.25	26.87	0	14.05	6.045	2.932	5.347	3.257	0.266
12.5	10.1	11.34	47.27	11.64	26.22	10.000	2.182	1.186	2.397	1.458
25	20.97	21.44	52.93	22.9	51.35	6.447	3.924	3.990	4.429	3.385
50	37.09	42.27	69.9	33.01		4.541	0.700	2.915	3.705	

evaluation 80% w/v of sugar intake were given to rats prior to hypoglycemic evaluation for the extracts.

The result of the oral glucose tolerance test of group A1 (treated 80% w/v of sucrose solution as a negative control) compared to group A2 (treated 80% w/v of sucrose and acarbose solution as a positive control) showed that the blood glucose concentration of the rats treated with 80% w/v sucrose solution is higher compared to treated with acarbose solution. Statisitic evaluation the difference at α 0.05, blood-glucose concentration after 0.5 and 1 h treatment of 80% w/v sucrose solution (Table 4).

The increasing of blood-glucose concentration was resulted by the absorption of glucose after the hydrolyzation of sucrose by sucrase enzyme to become glucose and fructose in the intestine. By acarbose treatment, this hydrolyzation process was inhibited and the inhibition of enzymatic activity was likely a competitive reversible reaction.

The hypoglycemic activity of the boiled water extract showed that the blood-glucose concentrations of B1 group (treated rats as negative control) were significantly higher ($\alpha = 0.05$) than those of B2, B3 (group of rats treated of boiled water extracts of *P.macrocarpa* old fruits pericarp with the doses of 6.20×10^{-3} mg g^{-1} w/w and 12.4×10^{-4} mg g^{-1} w/w of rats, respectively) and A2 (treated rats as positive control) groups (Table 5). The result showed that the boiled water extract of the old fruits pericarp decreased the blood-glucose concentration of rats treated with 80% w/v sucrose solution. The data from the experiment showed that at 0.5 and 1 h after treatment, the boiled water extracts of old fruit pericarp at the doses of 6.2×10^{-4} mg g^{-1} and 12.4×10^{-4} mg g^{-1} w/w of rats decreasing the blood-glucose concentration of rats after treated orally with 80% w/v sucrose solution significantly indifferent to those of acarbose with dose of 1.00×10^{-3} mg g^{-1} w/w of rats.

The hypoglycemic activity of the *n*-butanol part of the extract after 0.5 h treatment showed that the C1 group (treated rats as negative control) having blood-glucose concentrations higher (at $\alpha = 0.05$) compared to C3 (group of rats treated of *n*-butanol part extract of *P.macrocarpa* old fruits pericarp with the dose of 3.62×10^{-3} mg g^{-1} w/w of rats) and A2 (treated rats as positive control) groups, and showed significantly indifferent to those of C2 group (group of rats treated of *n*-butanol part extract of *P.macrocarpa* old fruits pericarp with the doses of $1.81\text{x}10^{-3}$ mg g^{-1} w/w of rats) (Table 6). After 1 h treatment, the blood-glucose

Table 3: Oral glucose tolerance test curve for determination of sucrose per oral for rats prior to hypoglycemic evaluation of the extracts. Group 1, 40% w/v; Group 2, 60% b/v and Group 3, 80% w/v, of sucrose solution given per oral

	Blood glucose rate (mg dL^{-1})			Standard deviation		
Time (h)	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
0	79.00	83.00	92.00	3.606	7.000	2.000
0.5	94.00	99.67	120.33	4.583	7.767	5.033
1	107.00	112.67	139.00	6.557	3.786	5.196
2	93.33	97.33	116.33	4.041	7.095	6.658
3	80.33	89.33	108.67	4.933	8.083	6.807

Table 4: The oral glucose tolerance test of group A1 (treated 80% w/v of sucrose solution as a negative control) compared to group A2 (treated 80% w/v of sucrose and acarbose solution as a positive control)

	Blood glucose rate (mg dL^{-1})		Standard deviation	
Time (h)	Group A1	Group A2	Group A1	Group A2
0	87.2	89.0	7.259	2.915
0.5	120.6	106.2	3.362	7.727
1	129.4	116.0	2.608	2.345
2	111.6	112.0	7.127	1.225
3	99.0	97.0	3.391	5.099

Table 5: Oral glucose tolerance test of rats. B2 and B3 (group of rats treated of boiled water extracts of *P. macrocarpa* old fruits pericarp with the doses of $6{,}20\times10^{-4}$ mg g^{-1} w/w and 12.4×10^{-4} mg g^{-1} w/w of rats, respectively) compared to B1 group (treated rats as negative control) and A2 group (treated rats as positive control)

	Blood Glucose (mg dL^{-1})				Standard deviation			
Time (h)	Group B1	Group B2	Group B3	Group A2	Group B1	Group B2	Group B3	Group A2
0	89.0	91.0	90.8	89.0	5.404	8.485	7.855	2.915
0.5	121.8	111.4	103.6	106.2	4.324	5.771	11.971	7.727
1	136.8	120.6	112.0	116.0	4.868	7.765	10.654	2.345
2	117.6	109.2	105.6	112.0	5.030	6.496	11.632	1.225
3	107.8	97.4	102.2	97.0	6.301	7.765	11.692	5.099

Table 6: Oral glucose tolerance test of rats. C2 and C3 (group of rats treated of *n*-butanol part extract of *P. macrocarpa* old fruits pericarp with the doses of 1.81×10^{-3} mg g^{-1} and 3.62×10^{-3} mg g^{-1} w/w of rats, respectively) compared to C1 group (treated rats as negative control) and A2 group (treated rats as positive control)

	Blood glucose (mg dL^{-1})				Standard deviation			
Time (h)	Group C1	Group C2	Group C3	Group A2	Group C1	Group C2	Group C3	Group A2
0	92	98	91	91	7.563	12.518	6.986	2.915
0.5	121	116	110	105	3.701	5.495	4.690	7.727
1	138	120	118	110	4.980	1.673	3.564	2.345
2	117	110	106	111	3.271	5.683	5.070	1.225
3	104	100	97	98	9.338	5.020	4.301	5.099

concentration of C1 group of rats having blood-sugar concentrations higher (at $\alpha = 0.05$) compared to those of C2, C3 and A2. This data showed that the *n*-butanol part extract of the old fruits pericarp at the doses of 1.81×10^{-3} mg g^{-1} and 3.62×10^{-3} mg g^{-1} w/w of rats decreasing the blood-glucose concentration of rats after treated orally with 80% w/v sucrose solution significantly indifferent to those of acarbose with dose of 1.00×10^{-3} mg g^{-1} w/w of rats.

Because the dose for water extract, *n*-butanol extract and acarbose given to the rats were converted from the dose uses for human, the results indicated that drinking 1-2 glass of water extract (from 5-6 dried of fruit pericarp boiled in 5 glasses of water until become 3 glasses) or 1-2 doses of butanol extract from 2 pericarp might gave similar hypoglycemic effect as 1 tablet of Glucobay (50 mg acarbose) for adult person.

The result of this experiment showed that the extracts of *P. macrocarpa* young and old fruits pericarp have hypoglycemic activity by *in vitro* experiment to inhibition of alpha-glucosidase enzyme and by *in vivo* experiment in the rats, therefore suitable with using it as traditional anti-diabetic drug. For the further investigation it is important to isolate pure chemical compound that content in the extracts of *P. macrocarpa* young and old fruits pericarp and which have alpha-glucosidase inhibition and characterized it by using spectroscopy (UV, IR, NMR, GC-MS). Besides it is important to perform toxicity test and hypoglycemic activity test by *in vivo* in the diabetes rats.

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