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by Ignatius Srianta

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In vitro α -glucosidase inhibitory activity of *Monascus*-fermented durian seed extracts

^{1,2*}Srianta, I., ²Kusumawati, N., ²Nugerahani, I., ³Artanti, N. and ⁴Xu, G.R.

¹Center for Food and Nutrition Research, Widya Mandala Catholic University Surabaya, Jalan Dinoyo 42-44 Surabaya 60625, Indonesia

²Department of Food Technology, Widya Mandala Surabaya Catholic University, Jalan Dinoyo 42-44 Surabaya 60625, Indonesia

³Research Center for Chemistry, Indonesian Institute of Science, Kawasan PUSPIPTEK Serpong 15314, Indonesia

⁴School of Biotechnology, Jiangnan University, 1800, Li Hu Road, Wuxi, 214122, Jiangsu Province, China

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Abstract

Study on the *in vitro* α -glucosidase inhibitory activity of *Monascus*-fermented durian seed extracts was carried out by inoculating the spore suspension of *Monascus* sp. KJR2 into boiled durian seed cuts and then incubated at room temperature for 14 days. The durian seed (DS) and powdered *Monascus*-fermented durian seed (MFDS) were extracted with distilled water and ethanol. The extracts were analyzed for the *in vitro* α -glucosidase inhibition activity and total phenol content. The results show that DS and MFDS ethanolic extracts have α -glucosidase inhibitory activity with IC_{50} of 199.1 μ g/mL and 70.7 μ g/mL, respectively. Water extract of DS and MFDS have low α -glucosidase inhibitory activity (<10%). The total phenol contents of MFDS extracts were higher than that of DS extracts. MFDS is potential for diabetes mellitus management.

Keywords

α -glucosidase inhibition activity
durian seed
Monascus
total phenol

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Introduction

Traditionally, angkak is produced by solid state fermentation with *Monascus* sp. on rice as the substrate. Many studies showed that it grows on a wide variety of natural substrates i.e. corn, cassava, wheat, potato and adlay (Yongsmith *et al.*, 1998; Ganrong *et al.*, 1998; Carvalho *et al.*, 2007; Pattanagul *et al.*, 2008), and various agro-industrial residues i.e. rice bran, wheat bran, cassava bagasse, jack fruit seed and durian seed (Dufosse *et al.*, 2005; Babitha *et al.*, 2006; Srianta *et al.*, 2012a; 2012b). Researchers worldwide focus their attention on the health effects, such as for antidiabetes, anticancer, antiobesity and stimulating bone formation. The frequency of diabetes in the world population is high and still increasing. In the course of the metabolic disorder, the concentrations of blood glucose frequently differ from normal especially after food intake. A reasonable way to control the carbohydrate-dependent disease would be to limit intestinal carbohydrate digestion. α -glucosidase is the enzyme that catalyzes the cleavage of glycosidic bonds in oligosaccharides.

Compound that can inhibit the activity of this enzyme is considered having antidiabetes activity because it could help preventing postprandial hyperglycemia by decreasing the rate of carbohydrate degradation to glucose (Kim *et al.*, 2004). Some researchers showed that *Monascus*-fermented products have great potential as antidiabetes (Chen and Liu, 2006; Chang *et al.*, 2006; Shi and Pan, 2012).

Shi and Pan (2010) reported that different types of *Monascus*-fermented products (*Monascus*-fermented rice, *Monascus*-fermented adlay and *Monascus*-fermented dioscorea) reduced the blood glucose levels in diabetic rats. Some researchers reported that phenolic content of the *Monascus*-fermented products are higher than that of unfermented materials (Tseng *et al.*, 2006; Lee *et al.*, 2009; Shi *et al.*, 2011). On the other hand, many researchers showed that α -glucosidase inhibitory activity have positive correlation to their phenolic content (Yoshikawa *et al.*, 2001; McDougall *et al.*, 2005; Mai *et al.*, 2007; Ramamurthy *et al.*, 2010; Adefegha and Oboh, 2012). The aim of this research was to study the potency of *Monascus*-fermented durian seed on *in vitro*

*Corresponding author.

Email: srianta_wm@yahoo.com

α -glucosidase inhibitory activity.

Materials and Methods

Materials

Durian seeds were obtained from local durian seller. Durian seeds were stored in a freezer (-40°C) until used. *Monascus* sp. KJR2 was obtained from Center for Food and Nutrition Research, Widyadarmasurabaya Mandala Surabaya Catholic University. It was maintained on Sabouraud's Dextrose Agar (SDA) slant, preserved at 4°C and subcultured monthly. After *Monascus* sp. KJR2 was grown on SDA slants at room temperature (30°C) under static conditions for 14 days, 10 mL of sterile distilled water was added and the spores were scraped under aseptic conditions. 0.1 mL of the spore suspension was inoculated into Sabouraud's Dextrose Broth (SDB) and then was incubated at room temperature (30°C) for 10 days. It was used as starter to produce *Monascus*-fermented durian seed.

Monascus-fermented durian seeds production

Durian seeds were boiled in a CaCO₃ solution of 5% w/v for 10 min to remove the mucus. After the seed coat was peeled off, the seeds were cut into small size of 0.3 cm x 0.3 cm x 0.5 cm. A 50 g of small cut durian seed was transferred into 300 mL flask, mixed thoroughly, autoclaved at 121°C for 15 min, then left to cool to room temperature, inoculated with the spore suspension of *Monascus* sp. KJR2 and incubated at room temperature (30°C) for 14 days in static conditions (with manual shaking daily). *Monascus*-fermented durian seed were dried in an oven at 45°C for 24 hours, ground and analyzed for *in vitro* α -glucosidase inhibition activity.

Preparation of water and ethanolic extracts

One g of *Monascus*-fermented durian seed (MFDS) and durian seed (DS, as a control) were transferred into a 250-mL conical flask and mixed with distilled water or 70% ethanol at a ratio of 5 mL of solvent per gram of fermented matter. The content was mixed by shaking at 243 rpm for 1 hour, left to stand for 15 min, and then filtered through Whatman No 1 filter paper. The filtrate was dried at 45°C and then used for *in vitro* α -glucosidase inhibitor activity assay.

In vitro α -glucosidase inhibitor activity assay

The α -glucosidase inhibitory assay was conducted according to Artanti et al. (2012). Sample (0.1 mL) was added to a test tube containing 0.1 mL of 20 mM pNPG (p-Nitrophenyl α -D-glucopyranoside) and 2.2 mL of 100 mM phosphate buffer at pH 7.0, and

then incubated for 5 mins at 37°C. The reaction was initiated by addition of 0.1 mL of enzyme solution (1 mg, 1 mL) followed by 15 min incubation at 37°C. The reaction was stopped by addition of 2.5 mL of 200 mM Na₂CO₃. The absorbance of p-nitrophenol released from PNPg at 400 nm was measured with a spectrophotometer. Percentage of inhibition on the α -glucosidase activity was calculated by the equation: $[1 - (B/A)] \times 100\%$; whereas A is absorbance in the absence of sample and B is absorbance in the presence of sample. IC₅₀ value is denotes the concentration of sample required to inhibit 50% α -glucosidase activity.

Determination of total phenol content

Total phenol was determined according to Gupta and Prakash (2009). by using folin-ciocalteu analytical grade, Merck) reaction in basic condition and the absorbance measured spectrophotometrically at 765 nm. Gallic acid was used as a reference. Total phenol content of the extract was expressed as μ g Gallic Acid Equivalent (GAE)/mL.

Results and Discussion

Table 1 show the α -glucosidase inhibition activity of durian seed and *Monascus*-fermented durian seed. Water extract of DS and MFDS have low α -glucosidase inhibition activity, less than 10%. IC₅₀ of both water extracts were not detected. Different results were found on ethanolic extract. Ethanolic extracts of DS and MFDS have high α -glucosidase inhibition activity with IC₅₀ of 70.7 μ g/mL and 199.1 μ g/mL, respectively. Elsewhere, other researchers have also reported on the α -glucosidase inhibition activity of ethanolic extract obtained from the of *Barringtonia racemosa* Roxb. and *Paeonia lactiflora*, *Syzygium cumini* (Linn.) (Gowri et al., 2007; Choi et al., 2009; Kumar et al., 2012). The α -glucosidase inhibition activity of ethanolic extract of DS reflected that naturally durian seed contain substances with inhibition activity. Higher α -glucosidase inhibition activity of ethanolic extract of MFDS reflected that during fermentation, the *Monascus* produce metabolites, which could increase the α -glucosidase inhibition activity.

Table 2 shows the total phenol contents of DS and MFDS extracts. This finding was similar to results reported for other *Monascus*-fermented products (Tseng et al., 2006; Lee et al., 2009; Shi et al., 2011). Shi and Pan (2011) found that phenolic compounds in *Monascus*-fermented dioscorea are gallic acid, vanillic acid, caffeic acid, epicatechin and coumaric. Enhancement of total phenolic soluble in ethanol was higher than that of soluble in water. The phenolic

Table 1. α -glucosidase inhibition activity of durian seed and *Monascus*-fermented durian seed extracts

Extract	Concentration ($\mu\text{g/mL}$)	% Inhibition	IC ₅₀ ($\mu\text{g/mL}$)
DS water extract	50	5.9	n.d.
	100	6.9	
	150	6.7	
MFDS water extract	50	9.7	n.d.
	100	8.1	
	150	9.2	
DS ethanolic extract	50	27.1	199.1
	100	36.7	
	150	46.2	
MFDS ethanolic extract	50	35.3	70.7
	100	65.3	
	150	80.7	

IC₅₀ = concentration of extract with 50% of α -glucosidase inhibitory activity
 Note: n.d. = not determined

Table 2. Total phenol contents of durian seed and *Monascus*-fermented durian seed extracts

Extract	Total phenol content ($\mu\text{g GAE/mL}$)		Enhancement ($\mu\text{g GAE/mL}$)
	DS	MFDS	
Water extract	325.8	589.8	264.0
Ethanol extract	132.0	473.3	341.3

soluble in ethanol produced during fermentation possibly contribute to the α -glucosidase inhibition activity.

Conclusions

DS and MFDS ethanolic extracts have higher α -glucosidase inhibition activity than that of water extracts. DS and MFDS ethanolic extracts have α -glucosidase inhibition activity with IC₅₀ of 199.1 $\mu\text{g/mL}$ and 70.7 $\mu\text{g/mL}$, respectively. Hence, MFDS has potential for application in diabetes mellitus management.

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