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Antioxidant and α-Glucosidase inhibitory activity of formulated ethanol extract of red yeast rice and rice bran

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Abstract
This research aimed to study the antioxidant and α-glucosidase inhibitory activity from the formulation of ethanol extract of red yeast rice and rice bran as well as to analyze total phenolic and flavonoid content. Red yeast rice and rice bran were extracted with ethanol through liquid-liquid extraction with n-hexane, dichloromethane, ethyl acetate, and water. Extract with the best antioxidant and α-glucosidase inhibitory activity was then formulated for antioxidant and α-glucosidase inhibition test. The highest antioxidant activity was found in red yeast rice and rice bran with the ratio of 1:1, having total phenolic and flavonoid content of 36.68 mg GAE/g and 11.10 mg QE/g respectively. The highest α-glucosidase inhibitory activity was found in formulation 1:0 of red yeast rice and rice bran, with IC_{50} value of 20.86 µg/mL. Meanwhile, other formulation of red yeast rice and rice bran (3:1, 1:1, 1:3) had IC_{50} value of 57.21, 34.52, 42.36µg/mL respectively.

Keywords: red yeast rice, rice bran, antioxidant, α-glucosidase inhibition.

Introduction

Diabetes mellitus (DM) is a degenerative disease indicated by hyperglycemia due to the absence or failure of part of or the entire body insulin in converting blood glucose into glycogen. DM is currently treated in several ways, by controlling diet (reduction of carbohydrate intake, consumption of complex carbohydrates or foods with low glycemic index), by inhibiting enzymes which catalyze carbohydrates in digestive tract (α-glucosidase and α-amylase), repairing pancreatic beta cells which are responsible of insulin production, and/or by injecting the right dose of insulin into the body.

The compounds in red yeast rice and rice bran are expected to have antioxidant activity which may be able to repair the pancreatic beta cells and inhibit α-glucosidase and α-amylase in the digestive tract. The enzyme inhibition plays an important role in preventing hyperglycemia. Enzyme α-glucosidase hydrolyzes disaccharides and elevates blood glucose. Inhibiting α-glucosidase will in turn allow DM patients controlling their blood glucose [1].

Uncontrollable hyperglycemia promotes the formation of free radicals by accelerating the formation reactive oxygen species (ROS) which can initiate oxidative stress. Oxidative stress can inhibit glucose uptake in muscle cells and adipocytes, decreasing insulin secretion by pancreatic beta cells and playing an important role in the development of diabetic complications such as heart attack, cancer, and hypertension [2]. Hence, antioxidant becomes an alternative in preventing diabetic complications.

Jung *et al* (2015) [3] found that rice bran which contains γ-oryzanol can act as antihyperglycemic agent by stimulating PPAR-γ (Peroxisome Proliferator-Activated Receptor Gamma) activity and promoting glucose digestion in insulin-resistant cells by stimulating translocation of GLUT4 (Glucosa Transporter Type 4) from cytosol to cell's surface. PPAR-γ is an important receptor in lipid metabolism and glucose balance. Rice bran is also rich vitamin B complex (B1, B2, B3, B5 and B6), vitamin E (tocopherols and tocotrienols), carotenoids, essential fatty acids, dietary fiber, amino acids, polyphenols, minerals, and phytosterol [4].

The dominant antioxidant compound in rice bran was ferulic acid at 4.21 ± 2.38 mg/100 g dissolved dry matter and 116.1 ± 81.0 mg/100 g undissolved dry matter, while total phenols was found at 596.3 ± 308.5 mg GAE/ 100 g dry weight [5]. Tiwari *et al* (2011) [6] identified proglycemic and antihyperglycemic activity of antioxidant-rich fraction of rice bran. Using DPPH method, they revealed that methanol extract of rice bran showed antioxidant activity of 45% and could inhibit α-glucosidase with IC_{50} value of 47.5 µg/mL.

Red yeast rice (*Angkak*), rice fermented by *Monascus purpureus*, is also reported to show

blood glucose reducing effect in diabetic patients. Solution of the fermented rice extract at two different level of dosage significantly reduced blood glucose [7]. Flavonoids, phytosterols, and pyrrole compound are potential to reduce blood glucose and triglyceride level when HDL-C rise. This was also seen in treatment against diseases due to metabolic syndrome [8]. Traditionally-fermented rice was proven to have active compounds such as those which resembles statin in structure, unsaturated fatty acids, sterols, and vitamin B complex [9].

Red yeast rice and rice bran have been reported to contain antioxidant and antidiabetic compounds. However, whether the two work synergistically or antagonistically as antioxidant and antidiabetic agent, as well as in their total phenolic and flavonoid content remain unknown. Hence, this research aimed to study the antioxidant activity and α-glucosidase inhibition of the formulated ethanol extract of red yeast rice and rice bran, as well as to determine their total phenolic and flavonoid content.

Materials and Methods

Preparation of red yeast rice samples and rice bran [10, 11]

Samples were dried, red yeast rice was put in an oven at 50ºC for 6 h, followed by grinding and sieving with 40 mesh sieve. Rice bran (40 mesh) was heated in an oven at 150 ° C for 10 minutes and then cooled at room temperature for 30 minutes.

Extraction of red yeast rice and rice bran [10, 11]

Red yeast rice powder (20 grams) was extracted by maceration with 400 mL ethanol 95%. The suspension was shaken for 3 hours at 130 rpm. Sample was filteres and concentrated with a rotary evaporator and then re-macerated in order to obtain ethanol 95% extract of red yeast rice.

Rice bran A (5 grams) was extracted with 20 mL of ethanol 95% by shaker at room temperature for 3 hours. The extract solution was then filtered, while the residues were extracted back twice. Extracts were then dried with a rotary evaporator at a temperature of 50°C.

Fractionation of ethanol extract of red yeast rice and rice bran

Fractinational of ethanol extract of red yeast rice and rice bran were determined essentially according to the method described by Ismail *et al* [10]. And Hasanah *et al* [11]. The ethanol extract of red yeast rice and rice bran were fractionated by liquid-liquid extraction using solvents with decreasing polarity, namely water, ethyl acetate, dichloromethane and n-hexane. The layers formed through fractionation were concentrated with rotary evaporator.

Formulation of Red Yeast Rice and Rice Bran Extract

The extracts used in the formulation were those with the best IC_{50} value in the α-glucosidase inhibition test [10]. They were ethanol extract of red yeast rice and rice bran. Five different ratio of ethanol extract of red yeast rice and rice bran were used in the formulation, 1:0, 3:1, 1:1, 1:3 and 0:1.

Assay of enzyme α-glucosidase inhibition [10]

The formation of red yeast rice and rice bran extracts were analyzed α-glucosidase inhibition. This assay used a microplate reader with p-nitrophenyl-α-D-glucopyranoside (p-NPG) as substrate and the enzyme α-glucosidase. As a positive control, acarbose solution was treated the same as the samples. The percentage of inhibition against α-glucosidase calculated to determine the IC_{50} value.

Samples were analyzed antioxidant activity with DPPH method and total flavonoid [12]. Total phenolic content was determined with Folin Ciocalteu method referring to study by Vongsak *et al.* [12].

Results and Discussion

Antioxidant Activity, Total Flavonoids, and Total Phenols

Table 1: Antioxidant activity, total flavonoids, and total phenols of formulated ethanol extract of red yeast rice and rice bran

Formulation of Red Yeast Rice and Rice Bran Extract	DPPH (µg/mL)	Total Flavonoids (mg QE/g)	Total Phenols (mg GAE/g)
Red yeast rice : rice bran (1:0)	37.83±0.00	13.95±0.00	34.78±0.00
Red yeast rice : rice bran (3:1)	9.51±0.31	5.18±0.00	43.48±0.01
Red yeast rice : rice bran (1:1)	5.95±5.53	11.10±0.01	36.68±0.00
Red yeast rice : rice bran (1:3)	6.39±0.61	15.77±0.00	58.15±0.02
Red yeast rice : rice bran (0:1)	29.27±6.69	20.92±0.01	47.83±0.02
Ascorbic acid	4.75±0.61	Quercetin	Gallic Acid

The results (Table 1) showed that the best formulation for antioxidant test was formulation 1:1 with the highest IC_{50} value of all (5.95±5.53 µg/mL). This IC_{50} value was not significantly different from that of ascorbic acid as positive control treatment (4.75±0.61 µg/mL). Meanwhile the lowest IC_{50} value was found in formulation 1:0 (100% red yeast rice) and 0:1 (100% rice bran). It indicated that molecules in red yeast rice and rice bran work synergistically as antioxidant.

Generally, analysis on total phenols showed no sign of synergism with the formulation, only as the effect of addition. Rice bran was increased in total phenols and flavonoids compared to red yeast rice. However, when they were combined at ratio 1:1, the total phenols was the average of that of red yeast rice and rice bran. The highest total phenols was found in formulation 1:3 at 58.15 mg GAE/g. It was followed by formulation 0:1 (100% rice bran), 3:1, 1:1, and 1:0 at 47.83 (mg GAE/g), 43.48 mg GAE/g, 36.68 mg GAE/g and 34.78 mg GAE/g respectively.

Analysis on total flavonoids revealed that formulation 0:1 (100% rice bran) had the highest total flavonoids with 20.92 mg IQE/g, while formulation 3:1 had the lowest one with 5.18 mg IQE/g. In their study, Rao *et al.* [13] reported that methanol extract of Njavara (India) rice bran had total phenols of 12.72 mg GAE/g, total flavonoids of 8.51 mg IQE/g, and antioxidant activity of 30.85 µg/mL. Meanwhile, Vasumathi rice bran was reported to have total phenols of 31 mg GAE/g, total flavonoids of 1.68 mg IQE/g, and antioxidant activity of 87.72 µg/mL. It seems that total phenols and flavonoids is positively correlated to antioxidant activity with DPPH method.

From the total phenolic and flavonoid content, it can be inferred that the presence of phenolic and flavonoid compounds do not necessarily increase the antioxidant activity. Furthermore, the data showed that ethanol extract of rice bran contributes more to the total phenol and flavonoid content than the ethanol extract of red yeast rice does.

α-Glucosidase Inhibition of Red Yeast Rice and Rice Bran Extract

Analysis on the inhibitory activity of red yeast rice and rice bran was carried out using enzyme α-glucosidase from

Bacillus stearothermophillus recombinant with substrate p-nitrophenyl-α-D-glucopyranoside (p-NPG). The inhibitory activity from the formulation was determined from their IC_{50} value (Table 2).

Table 2: α-Glucosidase inhibition of formulated red yeast rice and rice bran

Formulation of Red Yeast Rice and Rice Bran Extract	IC_{50} value (µg/mL)
Red yeast rice : rice bran	20.86
Red Yeast rice : rice bran (3:1)	57.21
Red Yeast rice : rice bran (1:1)	34.52
Red Yeast rice : rice bran (1:3)	42.36
Red Yeast rice : rice bran	26.39
Acarbose	0.19

Table 2 showed that: 1. Red yeast rice extract had better inhibitory activity toward α-glucosidase than rice bran extract, 2. There was no molecular synergism between the two extract, instead a possible antagonism, for formulation 3:1, 1:1 and 1:3 of red yeast rice and rice bran had worse IC_{50} value than that of when they worked alone, 3. The inhibitory activity of red yeast rice was closer to that of acarbose as positive control, although there was a big gap in IC_{50} value (20.6 and 0.1 µg/mL).

The enzyme inhibitory activity of red yeast rice was in agreement with report by Srianta *et al*. [14]. They reported that red yeast rice had potential in inhibiting α-glucosidase for it was fermented by *Monascus sp,* causing it to be rich in in phenolic compounds such as gallic acid, vanillic acid, epicathecin, and coumarin [14]. The derivative products of monascus pigment and penicillamine (H-Pen) and found that it inhibited α-glucosidase with IC_{50} value of 50.9 µM [15]. Meanwhile, Gomathi *et al.* [16] reported that ethanol extract of *Evolvulus alsinoides* had the best IC_{50} value of 86 µg/mL. Durian seed fermented by *Monascus sp* gave IC_{50} value of 70.7 µg/mL. Among them all, ethanol extract of red yeast rice in this study showed the best inhibition.

Based on its enzyme inhibitory activity, rice bran is no better than red yeast rice. Given that rice bran is not a fermentation product, its compounds are not as complex as those in red yeast rice extract.

Conclusion

The highest antioxidant activity of ethanol extract of red yeast rice and rice bran was found at formulation 1:1 with IC_{50} value of 5.95 µg/mL, total phenols of 36.68 mg GAE/g, and total flavonoids of 11.10 mg QE/g respectively. Meanwhile, the highest α-glucosidase inhibition was found at formulation 1:0 (100% ethanol extract of red yeast rice) with IC_{50} value of 20.86 µg/mL.

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