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Riceberry bran as a potential source of anti-diabetic agents and its Riceberry bran powder

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ABSTRACT

This study evaluated the anti-α-glucosidase effect, antioxidant activity, and total phenolic compounds of Riceberry bran extracts prepared by the extraction of Riceberry bran with distilled water, ethanol, or hexane using a maceration technique. The ethanolic extract resulted in the significantly highest α-glucosidase inhibitory activity, at 64.13±1.09%. The highest levels of antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radicals were found in the aqueous extract with a half-maximal inhibitory concentration (IC_{50}) of 0.26±0.001 and 0.13±0.001 mg/mL, respectively. Likewise, the total phenolic compounds of the aqueous extract were the highest at 36.20±1.80 mg GAE/g of extract. The Riceberry bran powder obtained by double drum drying was also examined for its physical and chemical properties. It was found that the Riceberry bran powder was of good quality and provided high levels of α-glucosidase inhibition (20.24±0.47%), antioxidant activities (IC_{50} for DPPH = 4.19±0.08 mg/mL, IC_{50} for ABTS = 1.57±0.07 mg/mL), and total phenolic compounds (31.25±1.32 mg GAE/g of powder). The overall results indicated the potential of Riceberry bran to provide α-glucosidase inhibitors, antioxidants, and phenolic compounds, which can be used as useful health promoters and nutraceuticals to combat diabetes, and its powder also had potential in the functional food industry.

Contribution/Originality: In this study, the organic Riceberry bran from the Organic Rice Production Group in Noun Sung sub-district, Yang Talat district, Kalasin, Thailand, was investigated for its pharmaceutical properties regarding Diabetes mellitus as well as its application as Riceberry bran powder.

1. INTRODUCTION

Diabetes mellitus (DM) is a non-communicable illness that has a major negative impact on people's quality of life, lifespan, and socioeconomic development globally. It also increases the financial strain on healthcare systems. According to the IDF Diabetes Atlas published in 2021 by the International Diabetes Federation (IDF), around 537 million persons worldwide between the ages of 20 and 79 have diabetes, making up approximately 10.5% of the adult

population (International Diabetes Federation, 2021). In addition, in 2021, diabetes caused 6.7 million deaths worldwide and cost 966 billion USD in medical expenses. It is projected that by 2030, there will be 643 million more people worldwide who suffer from diabetes (World Health Organization, 2021).

DM is characterized by increased blood glucose levels caused by insulin malfunctions, such as insufficient insulin secretion, action, or both (American Diabetes Association, 2013). One effective therapeutic approach is to delay the breakdown and absorption of glucose by inhibiting carbohydrate-hydrolyzing enzymes, particularly α-glucosidase (Kim, Jeong, Wang, Lee, & Rhee, 2005; Mcdougall & Stewart, 2005; Zhang, Wang, Dong, & Medicine, 2015). The α-glucosidase is a carbohydrate-hydrolyzing enzyme located at the epithelium of the small intestine and is a key enzyme that modifies postprandial hyperglycemia. Stopping α-glucosidase works to make the digestion and absorption of carbohydrates last longer, which effectively controls post-meal hyperglycemia (Dirir, Daou, Yousef, & Yousef, 2022; Kashtoh & Baek, 2022). It affects certain parts of cells, and it is believed that the problems associated with DM are due to an imbalance between the production of free radicals and the ability to eliminate them (Hao, Wang, & Lv, 2017). Flavonoids, phenolic compounds, terpenes, saponins, and cardiac glycosides are some of the naturally occurring antioxidants that protect against oxidative stress and free radicals (Ryan, 2011; Saji, Francis, Schwarz, Blanchard, & Santhakumar, 2019). Therefore, the most effective strategy to stop the oxidative destruction of various biomolecules is to consume enough of these biologically active substances, which are produced from plants, either directly or as a dietary supplement. Consequently, research aimed at discovering natural anti-diabetic substances has recently attracted increasing attention.

Rice (*Oryza sativa*) serves as both an economic commodity and a fundamental dietary staple in numerous countries. Global rice production amounts to approximately 680 million tons annually (Friedman, 2013). Thailand, specifically, contributes 25 million tons to this annual yield. Riceberry, an innovative type of black-purple rice, results from the crossbreeding of Hom Nil rice (a Thai non-glutinous purple rice) and Khao Dawk Mali 105 (Thai Hommali rice). This particular variety has gained popularity among Thai rice types due to its distinctive appearance, high nutritional value, and various health benefits (Min, Gu, McClung, Bergman, & Chen, 2012; Posuwan et al., 2013; Prangthip et al., 2013). It contains a high level of anthocyanins and phenolic compounds, therefore offering antioxidant activity to consumers (Jangmesin, Rimkeeree, & Tadakittisarn, 2017; Sivamaruthi, Kesika, & Chaiyasut, 2018). Furthermore, Riceberry bran, a by-product of rice milling, is also very nutritious because it contains high levels of anthocyanin, carotenoids (β-carotene and lutein), polyphenols, flavones, tannins, and catechins. Several studies have shown the chemoprotective properties, immune-enhancing activities, and antioxidant activities of Riceberry bran extracts (Arjinajarn et al., 2017; Arjinajarn et al., 2016; Min et al., 2012; Somintara, Leardkamolkarn, Suttiarporn, & Mahatheeranont, 2016).

In addition, Riceberry bran is also rich in dietary fiber, proteins, lipids, phytosterols, and many bioactive compounds, such as γ-oryzanol, ferulic, phytic, and γ-aminobutyric acids, tocopherols, and tocotrienols. These bioactive compounds are known for their health-promoting properties, including boosting immunity, being anti-inflammatory, nourishing the eyes, reducing fat accumulation in blood vessels, lowering blood pressure, inhibiting the growth of cancer cells, and increasing antioxidant activity (Esa, Ling, & Peng, 2013; Sivamaruthi et al., 2018). Although some previous studies revealed the effect of Riceberry bran oil supplementation to ameliorate hyperglycemia, hyperlipidemia, oxidative stress, and inflammation, their studies mainly focused on Riceberry bran oil. However, the information on Riceberry bran power extract, prepared by solvent extraction as an anti-diabetic agent, is limited. Therefore, this study was conducted to identify the anti-diabetic properties of Riceberry bran extracts prepared by solvent extraction regarding (i) the identification of α-glucosidase inhibition, (ii) the quantification of antioxidant activities, (iii) the assessment of total phenolic compounds, and (iv) the characterization of Riceberry bran powder in terms of physical and chemical properties. The knowledge gained from the present study would provide insight into the medical potential of Riceberry bran extract, which may be good for sustainable living by turning agricultural waste into a value-added product.

2. MATERIALS AND METHODS

2.1. Riceberry Bran Extraction

Riceberry bran was dried at 60°C in a hot-air oven and extracted with three solvents, including distilled water, ethanol, and hexane. The ratio of Riceberry bran to extraction solvent was 1:10, with extraction performed at 30°C on an orbital shaker at 200 rpm for 24 hours. The Riceberry bran extracts were separated by centrifugation at 12,000 rpm for 20 min and subsequently evaporated at 60°C using a rotary evaporator. Crude Riceberry bran extracts were kept at 4°C for further analysis. The yield of Riceberry bran extracts was expressed as a percentage and calculated according to the following equation:

$$Percentage\ yield\ (\%)\ =\ (A/B)\ x\ 100$$

Where A and B are the weight of crude Riceberry bran extract and the dry weight of Riceberry bran, respectively.

2.2. Determination of α-Glucosidase Inhibitory Activity

The method used to test α-glucosidase inhibitory activity was similar to that described by Kim et al. (2005), with a few small changes (Kim et al., 2005). Fifty microliters of Riceberry bran extract at a concentration of 10 mg/mL underwent preincubation with 1 unit/mL α-glucosidase in 0.1 M phosphate buffer, pH 6.8, for 10 minutes. Following preincubation, 400 µL of a 1 mM solution of p-nitrophenyl-α-D-glucopyranoside in 0.1 M phosphate buffer, pH 6.8, was introduced and incubated at 37°C for 30 minutes. Absorbance was then measured at 405 nm. The α-glucosidase inhibitory activity was denoted as the percentage of α-glucosidase inhibition and computed using the subsequent equation:

$$Percentage\ of\ inhibition\ (\%)\ =\ [(A-B)/A]\ x\ 100$$

Where A and B are the absorbance of a solution without Riceberry bran extract and the absorbance of a solution with Riceberry bran extract, respectively.

2.3. Determination of DPPH Radical-Scavenging Activity

We checked the antioxidants' ability to fight 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals using the method described by Brand-Williams, Cuvelier, and Berset (1995), with a few small changes (Brand-Williams et al., 1995). Riceberry bran extract, at various concentrations (50 µL each), was incubated with a 0.1 mM solution of 2,2-diphenyl-1-picrylhydrazyl radicals at 37°C for 30 minutes in darkness. After incubation, absorbance was measured at 517 nm. The DPPH radical-scavenging activity was quantified as the half-maximal inhibitory concentration (IC_{50}), representing the concentration of Riceberry bran extract that reduced the concentration of DPPH radicals by 50%.

2.4. Determination of ABTS Radical-Scavenging Activity

A procedure similar to that described by Re et al. (1999) was used to test the antioxidant activity against 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals, with a few small changes. Riceberry bran extract, in varying concentrations (50 µL each), was then incubated with the prepared mixture at 37°C for 30 minutes in darkness. Following incubation, absorbance was measured at 734 nm. The ABTS radical-scavenging activity was denoted as the IC_{50}, representing the concentration of Riceberry bran extract that reduced the concentration of ABTS radicals by 50%.

2.5. Determination of Total Phenolic Compounds

We measured total phenolic compounds using the Folin-Ciocalteu method, following the steps outlined by Singleton, Orthofer, and Lamuela-Raventós (1999), with a few small changes. In this procedure, 50 µL of Riceberry bran extract at a concentration of 10 mg/mL underwent preincubation with 500 µL 10% of Folin–Ciocalteu reagent at 37°C for 10 minutes. Subsequently, 1 mL of 7.5% sodium carbonate was added, and the mixture was further incubated for 1 hour at 37°C. Following incubation, absorbance was measured at 765 nm. The amount of total phenolic

compounds was measured in milligrammes of gallic acid equivalent (GAE) per gramme of extract. This was done by comparing the extract to a standard curve for gallic acid.

2.6. Preparation and Characterization of Riceberry Bran Powder

Distilled water was chosen for the preparation of Riceberry bran powder. Briefly, Riceberry bran was extracted by distilled water in the ratio of 1:10 at 80°C for 1 hour. Riceberry bran residue was removed, and Riceberry bran extract was then mixed with 5% maltodextrin and subsequently dried using a double drum dryer with the drum temperature at 130°C and the drum ratio speed at 1.0 rpm (Figure 1). The quality analysis of Riceberry bran powder was conducted regarding moisture content, water activity, color, α-glucosidase inhibitory activity, antioxidant activity, and total phenolic compounds.

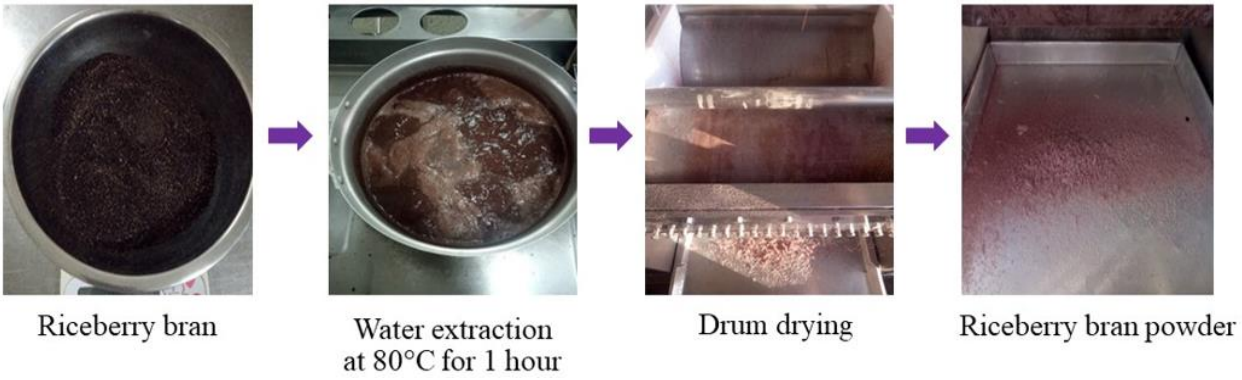

Figure 1. Process of preparing Riceberry bran powder.

2.7. Statistical Analysis

The data is presented as mean ± standard deviation. One-way analysis of variance (ANOVA) and least significant difference (LSD) tests were used to look at data that had a normal distribution. The significance level was set at $\alpha = 0.05$.

3. RESULTS

3.1. Riceberry Bran Extraction

Table 1 shows Riceberry bran extraction yields using distilled water, ethanol, and hexane. Hexane gave the significantly highest yield at 22.68±0.15%, followed by the ethanolic and aqueous extracts at 19.01±0.12 and 11.33±0.08%, respectively ($P<0.01$).

Table 1. Riceberry bran extract yields.

Riceberry bran extract	Yield (%)
Aqueous extract	11.33 ± 0.08^{c}
Ethanolic extract	19.01 ± 0.12^{b}
Hexane extract	22.68 ± 0.15^{a}

Note: Means with a,b,c different letter within a,b,c column are significantly different ($P<0.01$).

3.2. α-Glucosidase Inhibition, Antioxidant Activity, and Total Phenolic Compounds of Riceberry Bran Extracts

α-Glucosidase inhibition, antioxidant activity, and total phenolic compounds of Riceberry bran extracts were studied; the results are shown in Table 2. The significantly highest α-glucosidase inhibition was recorded for the ethanolic extracts at 64.13±1.09%, followed by the aqueous and hexane extract at 24.83±0.47% and 19.21±0.62%, respectively ($P<0.01$). The antioxidant activity of Riceberry bran extracts increased gradually as the concentration increased, indicating that the DPPH and ABTS radical-scavenging activities of the Riceberry bran extracts were

dose-dependent (Figure 2). The highest antioxidant activity of the Riceberry bran extracts was found in the aqueous extract, while the lowest IC_{50} values of the aqueous extract against DPPH and ABTS radicals indicated the highest antioxidant activities at 0.26±0.001 and 0.13±0.001 mg/mL, respectively ($P<0.01$). The ethanolic extract had an IC_{50} value of 0.88±0.04 mg/mL for DPPH radicals and 0.87±0.01 mg/mL for ABTS radicals. The hexane extract had the lowest antioxidant activity, with an IC_{50} value of 1.43±0.03 mg/mL for DPPH radicals and 1.94±0.07 mg/mL for ATBS radicals. The total phenolic compounds found in the Riceberry bran extracts corresponded to their antioxidant activities. The aqueous extract had the highest total phenolic compounds, being significantly higher than the other two Riceberry bran extracts at 36.20±1.80 mg GAE/g of extract ($P<0.01$), while the total phenolic compounds of the ethanolic and hexane extracts were 8.31±1.21 and 4.67±0.27 mg GAE/g of extract, respectively.

Table 2. α-Glucosidase inhibition, antioxidant activity, and total phenolic compounds of rice bran extracts.

Rice bran extract	α-Glucosidase inhibition (%)	IC_{50} (mg/mL)		Total phenolic compounds (mg GAE/g of extract)
		DPPH	ABTS	
Aqueous extract	24.83±0.47^b	0.26±0.001^c	0.13±0.001^c	36.20±1.80^a
Ethanolic extract	64.13±1.09^a	0.88±0.04^b	0.87±0.01^b	8.31±1.21^b
Hexane extract	19.21±0.62^c	1.43±0.03^a	1.94±0.07^a	4.67±0.27^c

Note: Means with a,b,c different letter within a,b,c column are significantly different ($P<0.01$).

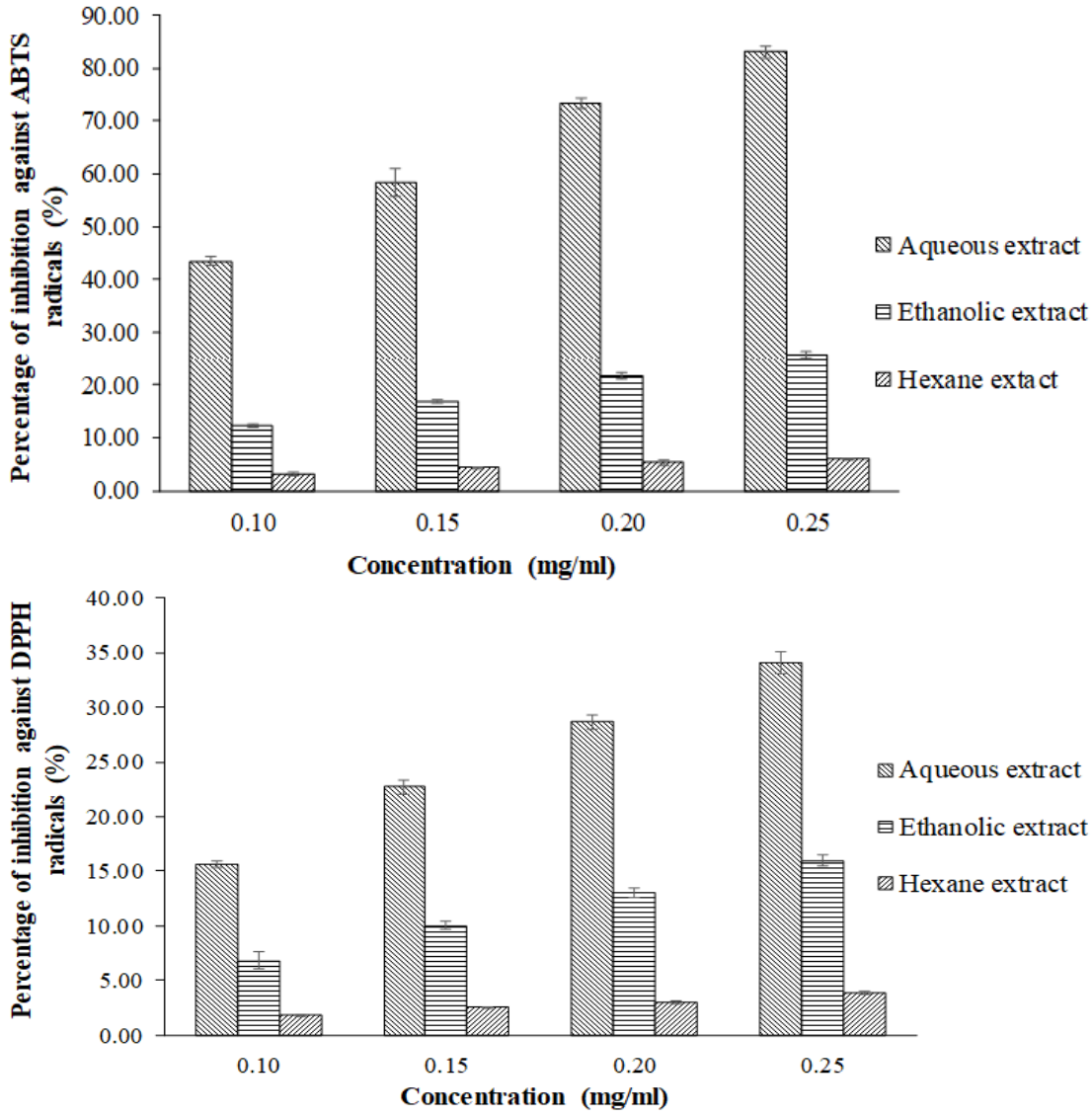

Figure 2. Antioxidant activities of Riceberry bran extracts.

3.3. Characterization of Riceberry Bran Powder

Table 3 shows the physical and chemical properties of Riceberry bran powder. The moisture content and water activity of Riceberry bran powder were 3.23±0.20% and 0.22±0.03%, respectively. The color of Riceberry bran powder was presented as L* (lightness), a* (redness), and b* (yellowness), with values of 45.80±0.26, 10.76±0.07, and 2.80±0.06, respectively. Riceberry bran powder still showed antioxidant activities against DPPH and ABTS radicals, with the IC_{50} as 4.19±0.08 and 1.57±0.07 mg/mL respectively. Its total phenolic content was 31.25±1.32 mg GAE/g of powder. The α-glucosidase inhibitory activity was 20.24±0.47%.

Table 3. Physical and chemical properties of Riceberry bran powder.

Parameters	Riceberry bran powder
Color	
L*	45.80±0.26
a*	10.76±0.07
b*	2.80±0.06
Moisture content (%)	3.23±0.20
Water activity (%)	0.22±0.03
IC_{50} for DPPH (mg/mL)	4.19±0.08
IC_{50} for ABTS (mg/mL)	1.57±0.07
Total phenolic compounds (mg GAE/g of powder)	31.25±1.32
α-Glucosidase inhibition (%)	20.24±0.47

Note: The L* indicates the lightness.
The a* indicates the redness.
The b* indicates the yellowness.

4. DISCUSSION

The rational for establishing different solvent extraction methods for Riceberry bran in this study was to find a suitable solvent to obtain high pharmaceutical properties from Riceberry bran. The different solvents with different polarities were tested in this study. The results indicated that using different solvents influenced both the yield and the biological activities of the Riceberry bran extracts. This finding was supported by several previous studies that revealed the importance of extraction solvents, their use in plant materials, and the extraction method used (Charirak & Ratananikom, 2022; Maisuthisakul & Changchub, 2014; Ratananikom & Premprayoon, 2022). In addition, factors including plant origin, plant genotype, geography, climate, soil fertility, and stress level could also have an effect on a variety of phytochemical components present in plants in quantity and form (Alabri, Al Musalami, Hossain, Weli, & Al-Riyami, 2014; Krishnaiah, Devi, Bono, & Sarbatly, 2009; Kubola, Chumroenphat, Meeinkuirt, & Weeradej, 2022). Therefore, in this study, solvents with a wide range of polarity from nonpolar to polar were used to ensure that all plant constituents, which were different in their structures and polarity, were extracted and available in the extracts. According to the results, hexane, as the solvent with the least polarity, offered a greater yield than ethanol and distilled water, solvents with higher polarity. This circumstance could be explained as a result of the composition of the Riceberry bran. Rice bran is a very good source of lipids, with lipid content ranging from 15.0% to 19.7% depending on the rice variety (Pereira, Lourenço, Menezes, & Brites, 2021). Two types of lipids have been reported in rice bran: (1) starch lipids, found inside the starch granules, which exist in a smaller proportion, and (2) non-starch lipids, found outside the starch granules, representing the majority (Champagne, 2004). As a result, low-polarity solvents like hexane were more effective at extracting these non-polar substances than ethanol and distilled water.

The Riceberry bran extracts also showed diverse nutraceutical properties regarding α-glucosidase inhibition. The ethanolic extract stopped α-glucosidase activity by 64%, which was three times stronger than the hexane and water extracts ($P<0.01$). This result reveals that different inhibitory effects resulted from the diverse components in each Riceberry bran extract. Plants are believed to synthesis various kinds of phytochemicals, which have an important role in plant metabolism and offer considerable health benefits to prevent diseases from occurring and slow the progression of diseases. Ethanol was the most suitable extraction solvent to obtain α-glucosidase inhibitors. This

result implied that the α-glucosidase inhibitors possibly consisted of functional groups that appeared to be hydrophilic with polarity indices of about 5.2 (absolute ethanol). According to the study by Yao, Sang, Zhou, and Ren (2010), it was found that Riceberry bran was high in polyphenols, especially anthocyanins. There are four anthocyanins available in purple and black rice, including cyanidin-3-glucoside, delphinidin-3-glucoside, petunidin-3-glucoside, and peonidin-3-glucoside (Yao et al., 2010). Cyanidin-3-glucoside is the main anthocyanin in coloured rice. It has been shown to stop α-glucosidase and α-amylase from working (Akkarachiyasit, Charoenlertkul, Yibchok-Anun, & Adisakwattana, 2010). Because of this, cyanidin-3-glucoside and other anthocyanins may have stopped α-glucosidase activity in rice bran extracts. In this study, an anti-α-glucosidase agent was not determined in Riceberry bran, but our data still suggested that Riceberry bran as a potential source.

The antioxidant activities and total phenolic compounds of the aqueous extract were the highest, in agreement with the results of Arab, Alemzadeh, and Maghsoudi (2011); Maisuthisakul and Changchub (2014); and Insuan, Chariyakornkul, Rungrote, and Wongpoomchai (2017), who indicated that rice bran contained many secondary metabolites such as polyphenols, vitamins, flavonoids, alkaloids, and phytosterols that could stop free radicals, thereby inhibiting or slowing down oxidative reactions (Arab et al., 2011; Insuan et al., 2017; Maisuthisakul & Changchub, 2014). In this study, distilled water was determined to be the most suitable solvent to obtain Riceberry bran extract with the highest antioxidant activity and total phenolic compounds, followed by ethanol and hexane, respectively. Different secondary metabolites formed hydrogen bonds with water and ethanol, but phytochemical substances reacted with hexane via hydrophobic interaction. Therefore, the main components in Riceberry bran extracts were different, leading to variance in their antioxidant activities and total phenolic compounds. All Riceberry bran extracts scavenged DPPH and ABTS radicals in a dose-dependent manner. This result is similar to the result of Oboh, Ademosun, Odubanjo, and Akinbola (2013), who revealed the concentration-dependent action of black pepper essential oils against DPPH and nitric oxide radicals and the chelation of Fe^{2+} (Oboh et al., 2013). Our results indicate that α-glucosidase inhibitors, antioxidant molecules, and phenolic compounds from Riceberry bane extract were susceptible to hydrophilic properties and were more likely to dissolve in polar solvents. These results concur with those of Yao et al. (2010); Luang-In, Yotchaisarn, Somboonwatthanaku, and Deeseenthum (2018); Jiang et al. (2017); and Brindis, González-Trujano, González-Andrade, Aguirre-Hernández, and Villalobos-Molina (2013). However, all data obtained from *in vitro* studies and, additional information *in vivo* regarding bioavailability and absorption should be determined before taking on their applications.

Due to safety concerns, practicality, and cost effectiveness, deionized water was chosen for the extraction process for preparing Riceberry bran powder. Physical properties were demonstrated to clarify the quality of Riceberry bran powder. Moisture levels and water activity can affect the shelf life of dried powder. To achieve a good quality of dried powder, the moisture content and the water activity of dried materials must be lower than 10% and 0.6%, respectively. In this study, the water activity of Riceberry bran powder was less than 0.6% and the moisture content was lower than 10%, indicating that it was suitable for storage for a long time and safe from spoilage caused by microorganisms. Color is a major quality parameter in a dried food product; color was presented in terms of L*, a*, and b*, which implied that the Riceberry bran powder was pink powder. These data agreed with the reports from Rittisak, Charoen, and Savedboworn (2022). Their study reported the optimal condition for broken Riceberry powder preparation as using a double drum dryer at 125°C and a drum speed of 1.0 rpm. Under their conditions, the broken Riceberry powder contained moisture content and water activity of less than 10% and 0.6%, respectively. Their broken Riceberry powder was also found as pink powder (Rittisak et al., 2022). The results on chemical properties obviously displayed that using a double drum dryer to prepare Riceberry bran powder did not demolish the activities on anti-α-glucosidase, antioxidants against DPPH and ABTS radicals, or phenolic content. The Riceberry bran powder still showed its pharmaceutical properties, which have potential for further development in the functional food industry.

5. CONCLUSION

In conclusion, Riceberry bran exhibited high nutraceutical potential to combat diabetes. The Riceberry bran extracts provided α-glucosidase inhibitory activity, which could suppress postprandial hyperglycemia, as well as being a good source of antioxidants and total phenolic compounds, which could help reduce oxidative stress in DM patients. The Riceberry bran powder also showed potential pharmaceutical properties. It had good quality and potential in the functional food industry.

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