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The Comparison of Antioxidant Activity and Acetylcholinesterase Inhibitory Activity of Unrefined Black Rice and Parboiled Germinated Unrefined Black Rice Extracts of Thai Local Rice (Mae Phaya Tong Dum)

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Abstract

Mae Phaya Tong Dum rice is the local black rice of Chanthaburi province, Thailand. This rice has the purple-black pigments presented in the rice bran. Black rice has reported that there is a good source of several bioactive compounds. This research emphasized the comparing of the antioxidant and anti-acetyl cholinesterase activities of unrefined black rice (UBR) and parboiled germinated unrefined black rice (PGUBR) extracts of Mae Phaya Tong Dum rice. The results found that UBR extracts obtained higher antioxidant and enzyme inhibitory activities than that of PGUBR extracts. The methanol extracted of UBR showed the highest antioxidant activity (IC_{50} = 217 µg/ml) and total phenolic content (469.716 µgGAE/g) more than that of methanol extracts of PGUBR (IC_{50} = 534 µg/ml and 334.489 µgGAE/g, respectively). Whereas hexane extracts were the best enzyme inhibitor, UBR had higher inhibition than PGUBR (IC_{50} = 58 and 181 µg/ml respectively). Using heat in the process of PGUBR from the Mae Phaya Thong Dam variety resulted in a decrease in the bioactivity of the black rice seed extract. Therefore, in the process of Germinated hang rice, it should be considered the heat used.

Keywords: Mae Phaya Tong Dum rice, Parboiled rice, Black rice, Anti-oxidant, Acetylcholinesterase

1. Introduction

Mae Phaya Tong Dum rice is a native black rice field to the Chong tribe, which is a traditional tribe in Chanthaburi province, Thailand. Consequently, the formal name of this rice is Khao Chong. After several years, this black rice had registered in the name of Mae Phaya Tong Dum rice. This rice has a purple-black in the bran layer and black in the tip of the grain or germ. The Chong tribe would boil this black rice plant to drink as a drinking water and folk medicine (1-2). Mae Phaya Tong Dum rice cultivated in Wang Saem, Makham, Chanthaburi, Thailand. Nowadays, this black rice variety is not popular to cultivate. Only some farmers plant Mae Phaya Tong Dum rice for conservation and consumption.

Black rice is the rice with a black bran layer. China and India were the largest black rice producer, followed by Thailand, Bangladesh, Vietnam and Indonesia, respectively. The black rice contains many essential nutrients, including starch, fiber, simple sugars, essential amino acids, proteins, lipids, vitamins and minerals. (3) Moreover, this rice also contained phenolic compounds, gamma oryzanol and, tocopherol. These bioactive compounds usually found in the bran and embryo of rice grains (3-4). Especially, anthocyanin and tricin were major flavonoids in black bran of rice (5).

Many researchers reported that black rice had more bioactive substances, nutrients and functional benefits than white rice. As a result, black rice had several biological activities, whether antioxidant activity (6-7) anti-inflammatory effect (8), anti-obesity (9), reduce the rate of starch digestion (10), anti-proliferation of cancer cells (11), promoting immune system in leukemia (12).

The bran layer of unrefined rice contains a lot of nutrients and biological activities. Thus, unrefined rice contains more beneficial nutrients than refined rice. Moreover, germinated unrefined rice has higher biological activities than unrefined rice and refined rice because the germination process of rice grains will produce a variety of bioactive metabolites (13). Despite claims of health benefits and nutraceutical values, the preference for unrefined black rice (UBR) remains low because of its unlikeable peculiar flavor, texture, and poor eating quality (14). Therefore, parboiled black rice was produced for solving the problem of texture and quality. (14-15)

The parboiled germinated rice also called Germinated Hang rice in some area. It was the traditional wisdom of the Phu Thai people, Sakon Nakhon province, Thailand. They used this process to increase the nutritional value of rice. (16) Moreover, it has been reported that when black rice undergoes this process, the eating quality of black rice was improved (14). Our research group interested in the comparison of the biological activities of unrefined black rice (UBR) and parboiled germinated unrefined black rice (PGUBR) extracts of Mae Phaya Tong Dum rice.

Alzheimer's disease causes brain cells to waste away (degenerate). The patients will lose their memory because the acetylcholinesterase (AChE) activity. The AChE breaks down the neurotransmitter acetylcholine causing a decrease progressively in the amount of this neurotransmitter. Consequently, the bioactive compound that inhibits the activity of AChE can slow or prevent Alzheimer's disease (17). Nowadays, everyone is at risk of developing health problems caused by free radicals and oxidation reaction from daily activities. It has been reported that rice extract showed antioxidant activity (6-7). Germinated unrefined rice prevented neurodegeneration and Alzheimer's disease (18-19).

The inhibition of the activity of acetyl-cholinesterase enzyme and antioxidation activities were used to assess the potential to reduce the risk of health problems and help to promote neurological health. Therefore, this research emphasized the comparing of the antioxidant and anti-acetyl cholinesterase activities of UBR and PGUBR extracts of Mae Phaya Tong Dum rice.

2. Materials and Experiments

2.1 UBR and PGUER preparation

Paddy black rice seeds and unrefined black rice (UBR) of Mae Phaya Tong Dum were collected from community store in Chanthaburi, Thailand. The paddy black rice used for parboiled germinated unrefined black rice (PGUER) by soaking in water for 24 hours, then wrapped up with a cloth and incubated until germination. After that, germinated rice was parboiled with the conventional method for 40 minutes and let the air dried. Finally, parboiled germinated unrefined black rice achieved using unrefined rice milling machine.

2.2 Extraction

UBR and PGUBR extracts were prepared from 1 kg of UBR and PGUBR powder rice seed, respectively. These samples were extracted using hexane, dichloromethane (DCM) and methanol (MeOH), successfully. Each extraction performed 3 days x 3 times, after that the organic solvent was evaporated using a rotary evaporator. 6 extracts, including hexane, DCM and MeOH extracts of UBR and hexane, DCM and MeOH extracts of PGUBR, were obtained.

2.3 Antioxidant activity assay

The 2,2-diphenylpicrylhydrazyl (DPPH) free radical scavenging assay is a rapid, simple, inexpensive and widely used method to measure the ability of compounds to act as free radical scavengers (20). The procedure taken as a measure of its antioxidant activity was determined based on the method of G.A.S. Premakumara et al. (21) with some modifications. Briefly, each extract was added in 96 wells plate with final concentrations ranging from 80 to 625 µg/ml and mixed with the 50 µl of 0.2 mM DPPH. The mixtures were kept in dark at room temperature for 30 minutes. After that, absorbance was read at 490 nm with the microplate reader. The scavenging of DPPH radical in percentage and half-maximal inhibition concentration (IC_{50}) were calculated by using butylhydroxytoluene (BHT) as a positive control.

2.4 Total phenolic content determination

Total phenolic content was performed according to D. Biedermann et al. (22) with some modifications. The standard gallic acid solution was prepared at a different concentration ranging from 41.8 to 66.8 µg/ml and each extract was prepared in distilled water 100 µg/ml. The reaction mixture contained 50 µl of standard solution or extract solution in distilled water was mixed with 1000 µl of Folin-Ciocalteau reagent diluted to 10%v/v in distilled water. After 5 minutes at room temperature, 1000 µl of 7.5% w/v of Na2CO3 solution was added. After the reaction mixture was kept in dark for 60 minutes at room temperature, the absorbance was measured at 725 nm using UV-Visible spectrophotometer. The total phenolic content was calculated as gallic acid equivalents per gram of extracts (µgGAE/g extracts)

2.5 Acetylcholinesterase inhibition assay

Acetylcholinesterase inhibition assay was carried out followed S.D. Giovanni et al. (23) with some modifications. Firstly, 300 µg/ml of standard rivastigmine solution in dimethyl sulfoxide (DMSO) was prepared from EXELON® (Rivastigmine tartrate 1500 µg) tablet and diluted to the final concentration ranging from 1.88 to 15,000 µg/ml. For sample test, each extract was diluted to the final concentration ranging from 30 to 2,000 µg/ml. After that, 5 µl of 0.003 M acetylcholine iodide was added followed with 5 µl of 0.0375 M that contained 0.01 M EDTA and the total volume adjusted to 80 µl with 0.1 M sodium phosphate buffer pH 7.4 that contained 1% v/v DMSO. The reaction was initiated by 20 µl of 1.4 unit/ml acetylcholinesterase (acetylcholinesterase from electric eel, Sigma-Aldrich). In the same way, the buffer was replaced an enzyme in blank. The plated was shaken for 5 minutes. Then, the absorbance was monitored at 405 nm using the microplate spectrophotometer. The inhibitory potencies of each sample were calculated in half-maximal inhibition concentration (IC_{50}).

2.6 Preliminary phytochemical analysis

For Preliminary phytochemical screening, S. Suwanjareon et al. methods (24) were used for testing alkaloids, saponins, terpenoids, anthraquinones and flavonoids. Ninhydrin reagent was used for testing amino acids and proteins. Whereas, oil was analyzed by the transparency of paper.

2.7 Statistical analysis

Each sample was analyzed in triplicate and standard deviations (S.D.) were reported. Data was analyzed for variance (One-way ANOVA, $p < 0.05$) and in the case of significance; means were compared using least significant different (LSD) ($p < 0.05$).

3. Results and Discussion

3.1 Extracts preparation

UBR and PGUBR were extracted using hexane, DCM and MeOH, successfully. For UBR extracts, % yield of hexane extracted higher than MeOH and DCM extracts respectively. (1.17, 0.99 and 0.50) For PGUBR extracts, %yield of hexane extracted (1.83) higher than DCM (0.92) and MeOH extracts (0.60). These results showed most of the chemical constituents of rice was non-polar. Hexane is a non-polar solvent. It will extract phytochemicals with the same polarity.

3.2 Antioxidant activity and total phenolic compound content

Six extracts from Mae Phaya Tong Dum rice were tested antioxidant activity using DPPH assay shown in table 1. MeOH extracts of PGUBR and UBR showed high antioxidant activity (IC_{50} = 534±0.016 and 217±0.040 µg/ml, respectively). MeOH is a polar solvent and extracts high polarity bioactive compounds. T. Oki et al. reported that the extracted using the high polar solvent showed better antioxidant activity than that of extracted using a non-polar solvent (25). These results were consistent with the results of the preliminary phytochemical screening. As shown in Table 2, MeOH extracted found several phytochemicals, including phenolic compounds, flavonoids, terpenoids, tannins, saponins, amino acids and proteins. These compounds are good antioxidants (4, 6, 26, 27), thus making MeOH extracted significantly better than DCM and hexane extracts ($p < 0.05$).

Moreover, MeOH extracted of UBR (IC_{50} value of 217±0.040 µg/ml) showed stronger antioxidant activity than that of PGUBR (IC_{50} = 534±0.016 µg/ml) ($p < 0.05$). Which corresponds to the total phenolic contents in UBR extracted more than PGUBR extracted (469.716 and 334.489 µgGAE/g, respectively). Phenolic compounds were good antioxidants (28), indicating that UBR extracted has better antioxidant activity than PGUBR. When the black grain was cooked with heat, the number of phenolic compounds, especially anthocyanins (which are the main bioactive compounds in black rice) was reduced. As a result, the antioxidant activity is reduced as well. In the same way with phenolic compounds, amino acids and proteins showed antioxidant activity. When it heated, it will lose its functional properties. From the above reasons, UBR had a higher total phenolic content and showed better antioxidant activity than PGUBR. These results were consistent with the research of M. Ang-Lopez et al. (14) which is reported that the uncooked black rice contained higher anthocyanin content and showed stronger antioxidant activity than boiled rice.

Table 1 Antioxidant activity using DPPH assay of extracts from Mae Phaya Tong Dum Rice

Samples	Solvent	IC_{50} (µg/ml)
PGUBR	Hexane	4,394±0.159^{b}
	DCM	3,167±1.641^{c}
	MeOH	534±0.016^{d}
UBR	Hexane	5,451±0.112^{e}
	DCM	4,251±0.142^{f}
	MeOH	217±0.040^{g}
BHT (Positive control)		27±0.002^{a}

$^{a, b, c, d, e, f, g}$ The mean was statistically significant difference at $p < 0.05$.

3.3 Inhibition of acetylcholine esterase activity

Acetylcholinesterase enzyme inhibitory activity of Mae Phaya Tong Dum rice extracted showed in Table 3. Hexane extract of UBR (IC_{50} = 58 ± 0.025 µg/ml) showed higher inhibitory activity than that of PGUBR (IC_{50} = 181±0.029 µg/ml). Because the hexane extracts were contained non-polar or low-polar phytochemicals such as rice bran oil, which is found in most of the rice bran layer. This is consistent with the phytochemical examination results in Table 2. Rice bran oil is a good source of many bioactive compounds including γ-orizanol that is reported to promote a wide range of health conditions, including its anti-anabolic activity, anti-inflammatory, stimulate the immune system (29) and prevent Alzheimer's disease (30). Besides, riceberry bran oil could provide beneficial effect on diabetes by decreasing oxidative stress and recovering organ histology (31).

Table 2 Preliminary phytochemical testing of extracts from Mae Phaya Tong Dum rice.

Phytochemical	PGUBR			UBR		
	Hexane	DCM	MeOH	Hexane	DCM	MeOH
Phenolic compounds	-	-	+	-	-	+
Alkaloids	-	-	-	-	-	-
Flavonoids	-	-	+	-	-	+
Anthraquinone	-	-	-	-	-	-
Terpenoids	+	+	+	+	+	+
Saponins	-	-	+	-	-	+
Amino acids and proteins	-	-	+	-	-	+
Oil	+	-	-	+	-	-

One of chemical constituents of all extracts is terpenoids. Some of terpenoids are non-polar and some of them have polarity. However, the terpenoids were reported to inhibit the acetylcholinesterase activity, which causes Alzheimer's disease (32-33).

Table 3 Acetylcholinesterase inhibition of extracts from Mae Phaya Tong Dum Rice

Samples	Solvent	IC_{50} (µg/ml)
PGUBR	Hexane	181±0.029[b]
	DCM	357±0.164[c]
	MeOH	1,278±0.210[d]
UBR	Hexane	58±0.025[e]
	DCM	164±0.028[f]
	MeOH	272±0.095[g]
Rivastigmine		4±0.001[a]

a, b, c, d, e, f, g The mean was statistically significant difference at $p < 0.05$.

4. Conclusions

From the antioxidant activity using DPPH assay of UBR and PGUBR of Mae Phaya Tong Dum rice, UBR extracts had bioactive compounds more than the extract of PGUBR. The MeOH extract of UBR showed the best antioxidant activity and contained a higher total phenolic content than DCM and hexane extracts, respectively. The primary phytochemicals of MeOH extract found highly polar groups such as phenolic compounds, flavonoids, saponins, terpenoids, amino acids and proteins.

Hexane extract obtained the best acetylcholinesterase inhibitory activity. The primary phytochemicals found non-polar groups such as oil and terpenoids. This research showed that germination process affected the biological activity of rice extract of Mae Phaya Tong Dum rice. Therefore, alternative methods should be used to improve the consumption quality of this rice cultivar or improve the process of germinated rice to maintain better bioactive substances. However, this research has shown that Mae Phaya Tong Dum rice having good nutritional value and contains bioactive compounds that help promote the health of consumers. Consequently, it should be promoted to increase consumption and cultivation.

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Declaration of conflicting interests

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