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Evaluation of Antioxidant Activity and Total Polyphenols Content on Upland Rice

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ABSTRACT

Upland rice is selected by health-conscious consumers, preferably for its unique taste and fragrance. However, the yield was lower compared to the white rice. Still, limited study on its chemical property as compared to commercialized rice has been done. Investigation of its chemical properties including antioxidants and polyphenols were carried out on the selected upland rice variants from selected regions in Malaysia. Two upland rice varieties labelled as Bukit Pulut and Bario Bukit were used together with two varieties of wetland rice; Mahsuri and Sri Malaysia 1 as a comparison. The results showed that upland rice variants showed higher antioxidant properties through the DPPH and ABTS assays as compared to the wetland rice variants. The total phenolic content of Bario Bukit was the highest followed by Bukit Pulut and the other two wetland rice. Nevertheless, for the total flavonoids content, the results were varied among the rice variants. Based on the study, two upland rice varieties were found to have better chemical properties (antioxidant and polyphenols) therefore this increased their nutritional value than the wetland rice varieties.

Keywords: Upland rice, Antioxidant property, Total polyphenols.

INTRODUCTION

Rice (Oryza sativa L.) is known as the predominant food and an important crop for human population throughout the world in addition to wheat and maize. Rice has different varieties, flavours and colours pigments that are consumed based on individual preferences. Although white rice has lost their natural wholeness through the refining and bleaching process; majority of the consumers choose white rice because of its appearance, taste, and aroma. On the contrary, pigmented rice such as brown rice (which restores bran and side hull) is rich in thiamine, magnesium, proteins, calcium, fibre, potassium and also has lower glycemic index [1]. Based on the geographical and climate condition of the planting area, there are two types of rice namely upland and wetland rice. Upland rice refers to rice that are cultivated under dryland conditions and relies entirely on rainfall or irrigation depending on the amount of precipitation the area received [2]. The cultivation is also limited to the climate and seasons, and it is usually planted in the rural area with acceptable milling process. Conversely, flooded rice, also known as wetland or waterlogged rice is grown on flatland in flooded soils [3]. Scientifically, rice remarkably contains phyto-components similar to the grains which include carbohydrates, proteins, certain fatty acids and micronutrients [4-8]. In the rice variants,

colored rice is the more favorable source due to the higher level of phenolics, vitamins, and antioxidants [7-9] in comparison to the white rice. Phenolics are major classes of phytochemical substances produced by majority of plants. The presence of phenolic compounds in rice is important for plant development and to protect theplant from diseases and infections. The existence of phenolic compounds including flavonoid and phenolic acid are also importance for grain characteristic of rice that is directed to the index of grain resistance [10]. Ferrulic acid, p-coumaric, caffeic acid, vanillic acid, syringic acid, chlorogenic and gallic acid are a part of the phenolic compounds in rice that has been reported by [4-6] and [11]. In addition, phenolics and their derivatives had also contributed to higher level of antioxidant [8,12]. The findings proved that brown rice that is rich in phenolic exerted good antioxidant property compared to white rice. Brown rice could be a good source of antioxidant and also the increased nutritional value.

These days, consumers tend to find an alternative food that is rich in nutritional quality to promote a healthier lifestyle. As rice is the staple food for most of the population, rice with higher nutritional as well as antioxidant capacity was selected. In term of oxidation, it is defined as reactive oxygen species or free radicals of unstable electron of molecules that occurs as a by-product of a metabolic pathway. The overproduction of the unstable electron on the living organism may cause the cells to suffer the consequences of oxidative stress and it is also related to disease such as hypertension and heart attack [13]. To delay the oxidation process, antioxidant substance is used to neutralize the free radical species. The antioxidant process can be done either by inhibiting the enzyme, electron donation, chelating trace elements and scavenged reactive oxygen species [14-15]. Natural compounds like polyphenols such as phenolics or other plants phytochemical substances could be used as an antioxidant to reduce the oxidation process occurring in the living cells [16-17].

Phytochemical investigation of the phenolic compounds and their antioxidant property on the rice variety are increasing nowadays. Until recently, phytochemicals and antioxidant property were established on brown rice samples [5,6,11,18], red rice [4, 8], white and wild brown rice sample [7]. However, the limitation of the current study is limited to the rice planted in wetland area. To the best of our knowledge, the phytochemical content and the antioxidant property of upland rice type is still limited. The existence of some phenols group in the brown and white rice could be similar to the upland rice, where it can be used as the general guideline in this study. The specific objectives of this study were to investigate the antioxidant property, total polyphenols content and phytochemical constituent of the polyphenols including the phenolic and flavonoids content from the upland variants. The contribution of the study is to obtain clear information on the upland rice as information on its antioxidant property and phenolic compounds are currently limited.

MATERIALS AND METHODS

Rice sample

The upland rice variants (brown rice) were taken from farmers from rural area located in the West Malaysia region. Two varieties of upland rice namely Bario Bukit was obtained from from Sarawak and Bukit Pulut was from Sabah. The upland rice samples were prepared in the traditional way to obtain the brown rice by separating the husk from the outermost layer without milling and polishing steps. As a comparison with the commercial rice, two wetland rice variants (white rice type); labelled as Mahsuri and Sri Malaysia 1 were obtained from the Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Malaysia.

The extraction procedure

The extraction process was carried out according to [8] with minor modifications. 2 g of grounded rice samples were extracted with 10 ml of 80% ethanol. The samples were shaken continuously for 3 hours at ambient temperature. The extracted samples were centrifuged at 2500 rpm for 10 minutes and the supernatants were collected. The extraction was continued, where the residue were shaken for 1 hour. These two supernatants were combined and dried using rotary evaporator before re-suspended in 5ml of 80% ethanol prior to analysis. The solutions were stored in the refrigerator at -25°C.

Antioxidant activity: DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay

The assayed was done according to the following procedure adopted from [8]. 100ul of the extract was mixed with 1.9 ml DPPH solution (0.1 mM in 80% ethanol) and the preparation was done under minimal light condition to preserve the DPPH radicals. The sample was incubated to allow the reaction at room temperature condition for 30 minutes before absorbance was read at 517 nm using UV-Vis spectrophotometer. The assays were done in triplicate measurements for both samples and the standard. DPPH solution was used as the positive control. The percentage of the scavenging activity was calculated using the following formula:

$$\% \text{ Scavenging activity} = 1 - (A_o/A_c) \times 100$$

Where:
A_o : absorbance of sample
A_c : absorbance of positive control

The inhibition to the scavenging activity of the 50% of DPPH radicals was calculated from the linear regression graph. As a comparison, a standard antioxidant compound; ascorbic acid was used.

Antioxidant activity: ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic) radical scavenging assay
The ABTS radical scavenging assay was done according to the method by [19] with some modifications. ABTS reagent was prepared using water (7mM). To produce the radical $ABTS^{\cdot+}$, 2.5mM potassium persulfate was mixed with ABTS reagent at the ratio of 1:1 and the solution was allowed to stand with minimal light and room temperature for 6 hours to complete the chemical reaction. The oxidation of $ABTS^{\cdot+}$ was stable for 2 days if it is stored in a dark place at room temperature. To perform the assays, the ABTS solution was diluted with ethanol and spectrophotometrically (using UV-Vis spectrophotometer) measured until it reached the value of 0.70 with a wavelength of 734nm. Freshly prepared sample and the standard (50µl) that was prepared at different concentrations were mixed with 1.5ml of $ABTS^{\cdot+}$ solution. After 5 minutes, the mixed solutions were measured at 734nm. The $ABTS^{\cdot+}$ solution was used as the positive control. The percentage of the inhibition of the samples and the standard were calculated through a concentration-response curve. The affinity of the test material to quench ABTS free radical was evaluated according to the following formula:

$$\% \text{ Scavenging activity} = 1 - (A_o/A_c) \times 100$$

A_o : absorbance of sample
A_c : absorbance of positive control
Fifty percent (50%) of the inhibition was calculated from the calibration curve and results were expressed in concentration unit (mg/L). Ascorbic acid was used as the reference for this assay.

P**olyphenol: Total Phenolic Content**
The Follin-Ciocalteu method was chosen to determine the total phenolic content of the rice variants, which was done according to procedure by [8] with minor modifications. Firstly, the sample (200ul) was mixed with 1 ml of Follin-Ciocalteu reagent (2N) and the solution was left for 5 minutes to allow the reaction to occur. To stop the reaction, 800 µl 10%Na_2Co_3 was added to the solution where a change of colour either becoming dark or light green depending on how strong or slow the reaction with the phenols. The solution was then diluted with 5 ml of distilled water and after 2 hours of leaving it in a dark place, the readings were measured using a Perkin Elmer UV-Vis spectrophotometer. The absorbance for phenols was measured at 760nm. As a comparison, a standard of gallic acid has been used as the reference and standard curve was prepared with different concentration of Gallic acid (mg/L). The total phenolic content was expressed as mg gallic acid equivalents per g of grain.

Polyphenol: Total flavonoid content
The total flavonoid content of the samples was determined by using the aluminum chloride assay method [20]. 1ml of the grain extract and the standard solutions were added with 1ml of 2% Aluminium Chloride and incubated for 15 minutes in a dark place. The absorbance of the samples was measured at 430nm using UV-Vis spectrophotometer (Perkin Elmer, Lambda 25). Rutin was used as the chemical reference and it was prepared at different concentrations to calculate the flavonoids content through a calibration curve and the results were expressed as mg Rutin equivalent (RE) per 1 g rice (mg RE/g).

Statistical analysis
The experimental data of the total polyphenols content and the antioxidant property of the upland rice samples were analyzed statistically through the analysis of variance (ANOVA) using Duncan Multiple Range test (DMRT). The p value was less than 0.05 at a significance level. The means of the samples were compared with the experimental data at three selected parameter.

RESULTS AND DISCUSSION

Rice could be a good source of nutritional food rich in antioxidants. The chemical assay using reactive free radical of DPPH and ABTS is frequently selected and assigned together to measure the antioxidant property on different ranges of plant samples including grain. The assay was chosen as; DPPH reaction is more suitable for a compound that has lipophilic character, meanwhile ABTS is more suitable for both hydrophobic and lipophilic character. The normal term of IC_{50} is normally used to describe the effect of scavenging activity at their effective concentration.

The lower concentration value of IC_{50} signified better antioxidant activity. In this study, the scavenging activity of the standard ascorbic acid was used as the scavenger indicator to measure the scavenging response within the rice samples. The results of the DPPH assay showed that ascorbic acid is capable of scavenging about 93.84±0.09% at a concentration below 1000 µg/ml with respective IC_{50} of 262.5 ± 0.4µg/ml (result not shown). In general, the rice variants were significantly different using both assays at the level at p> 0.05. Results on the antioxidant activity revealed that two upland rice variants served better scavenging effect when compared to both of the wetland rice variants. For upland rice variants, the IC_{50} values obtained was in ascending order from Bukit Pulut to Bario Bukit. However, the wetland rice signified lower antioxidant activity probably due to a lower presence of active antioxidant compound of the polished rice variant compared to the colored rice of the upland variants. Some of the plant nutrients were probably removed through the milling and bleaching process in order to exhibit white and aromatic rice. In another study by [21], the percentage of the scavenging activity of the selected upland rice genotypes from Malaysia was about 31.85-98.45% which is higher than the non-pigmented white rice. In accordance to the results obtained, the percentage of the scavenging activity that was measured at a concentration of 1000 µg/ml was within the acceptable range; 67.47% (upland rice Bario Bukit-Figure 1) and 43.83% (upland rice Bukit Pulut-Figure 1). However, the author does not clearly mention the effective concentration on the tested upland rice genotypes. In this study, the IC_{50} measured for the Bario Bukit is 810.47±7.46µg/ml, meanwhile for Bukit Pulut, the measurement obtained was 2250.34±30.03µg/ml. This could be explained where upland rice is also a type of pigmented brown rice that was reported to contain high nutrient and antioxidant property compared to the non-pigmented rice [5,7,8,22]. Also, [21] had evaluated upland rice variants which have higher content of micronutrients (Fe, Mn, Zn and Cu) and phenols, thus, this supported the findings of this study.

Table 1: The antioxidant activity of rice variants by ABTS and DPPH assays

Method	**DPPH**	**ABTS**
Sample/Response	IC50	IC50
Upland rice Bario Bukit	810.47±7.46a	3480.93±56.25a
Upland rice Bukit Pulut	2250.34±30.03a,b	3820.59±20.62a
Wetland rice Mahsuri	7835.05±45.53b,c	14910.69±633.28c
Wetland rice Sri Malaysia 1	8709.82±6466.28	27038.82±69.77d
Means with different letters were significantly different at level p<0.05 using DMRT.		

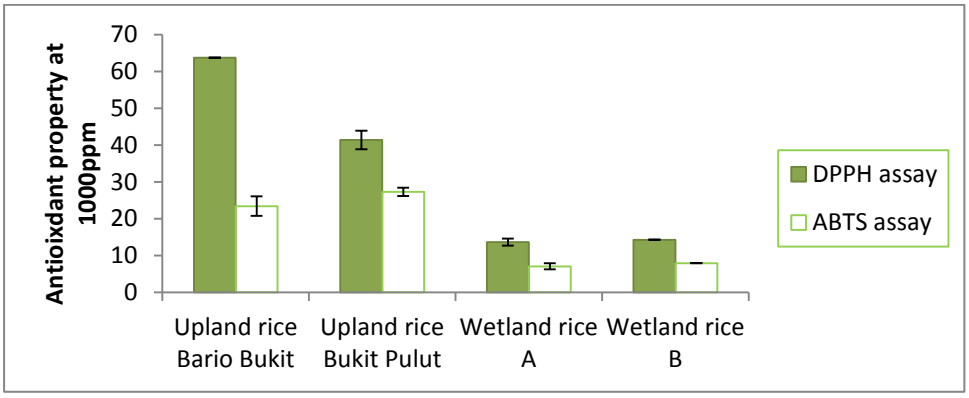

Figure 1: The antioxidant property at 1000 µg/ml among the rice variants from the DPPH and ABTS assay

Similar trends for upland rice variants were observed using ABTS assay as illustrated in Figure 1. Corresponding to the DPPH radical, ascorbic acid was also used as the reference and the calculated IC_{50}. Based on this assay, the response of the antioxidant radical nearly produced a similar response to the DPPH assay. However, a higher concentration is required to scavenge the oxidized molecules at its effective concentration. For example, the upland rice Bario Bukit sample was four times lower in their response towards ABTS compared to DPPH radical. Examination of all the rice samples indicated significant differences. The upland rice Bario Bukit and Bukit Pulut exhibited the strongest ABTS antioxidant activity at IC_{50} 3480.93 µg/ml and 3820.59µg/ml, respectively. It can be seen from the data that higher concentration is required to achieve 50% of scavenging effect for non-pigmented

wetland rice of A and B. It is apparent from Table 1 that similar trends of antioxidant activity were observed using both methods. Furthermore, it was believed that the processing and milling of the rice contributed to the decline of the antioxidant activity as well as the nutritional content of the rice. The antioxidant activity based on the ABTS radical assay was not evaluated so far in previous study of the upland rice sample. Therefore, this preliminary data can be referred for further investigation on the antioxidant and plant nutritional study.

Subsequent total phenolic content was measured in both rice variants ranged from 20.00 to 47.84 mg/g Gallic acid equivalents in dry grain weight (Table 2) where the upland rice variants showed the highest phenolic content. This phemenon ascertain the results obtained previously. The upland rice, Bario Bukit was significantly different where the result obtained was 47.84 mg/g GAE compared to the Bukit Pulut, 28.78 mg/g GAE. Similar result was obtained for the wetland rice variants which ranged from 20.00 to 28.19 mg/g GAE. The data from the present study are in accordance as reported by [6,7,11] who have claimed higher phenolic content in pigmented brown rice of compared to the white rice [8]. Even reported that the phenolic content of the red-colored wild rice species understudy is five times higher than the commercial white rice. Moreover, these existing reports recognized phenolic acids including ferulic acid, caeffic acid and syringic acid in rice grain that is similar to other grains [7,23,26]. Phenolic compound which is a known antioxidant components implicated various medicinal properties such as anticancer, anti-aging and anti-inflammatory effects [17,24]. Despite that, the data for phenolic content in the upland rice has not been reported except for pigmented rice. However, this study has proved that upland rice variants showed higher level of phenolic compound as compared to the white rice.

Table 2: The polyphenols content

Method	**Total phenolic content (mg/g)**	**Total flavonoid content (mg/g)**	**Ratio TFC/TPC (%)**
Sample/Response			
Upland rice Bario Bukit	47.84±0.31a	7.14±0.23a	14.92
Upland rice Bukit Pulut	28.78±0.28b	3.52±0.18b	12.23
Wetland rice Mahsuri	27.19±0.11d	6.36±0.01a	23.39
Wetland rice Sri Malaysia 1	20.00±0.35e	3.35±0.60b	16.75
Means with different letters were significantly different at level $p<0.05$ using DMRT.			

On the other hand, flavonoid which is supplementary for phenolic components such as rutin and quercetin are reported to contain higher antioxidant activity at lower IC_{50} concentration [25]. The amounts of the flavonoids compounds in the rice variant were varied due to the phenolic content in the rice grain. Based on the result (Table 2), it can be summarized that the ratio of the flavonoids content to the phenolic compound differs less than 15% in both the upland rice variants. Meanwhile, the wetland rice was examined between 16.75-23.39% of the ratio flavonoids to the phenolic content.

CONCLUSION

This study has chemically described the polyphenols content and antioxidant property as well as the specific phytochemical component of the upland rice variant in comparison with the wetland rice. As non-pigmented upland rice which has better nutritional aspects than the white rice, as the non-pigmented upland rice can serve as an alternative for a healthier lifestyles. However, limited access for plantation geographical factors is one of the challenges in commercializing the non-pigmented upland rice. In this study, the upland rice can be further highlighted due to its rich antioxidant content that reflects the high content of polyphenols and phenolic acid.

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