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Physical, antioxidant and antibacterial properties of rice (*Oryza sativa* L.) and glutinous rice (*Oryza sativa* var. glutinosa) from local cultivators and markets of Peninsular, Malaysia

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Abstract

Rice (*Oryza sativa* L.) is an important grain and basic food for a large population in the world, especially in Malaysia. It has been reported to contain highest antioxidative and antibacterial properties against pathogenic bacteria such as *Bacillus cereus* that may cause diarrhoea and vomiting in human bodies. Therefore, ten rice (*Oryza sativa* L.) (four of pigmented and six of non-pigmented) and four glutinous rice (*Oryza sativa* var. glutinosa) varieties (two of pigmented and two of non-pigmented) from local cultivators and markets of Peninsular, Malaysia were studied for their colour parameters, antioxidant and antibacterial properties. The pigmented rice samples showed the highest antioxidative properties such as total phenolic content (TPC) (11.12±2.49 – 104.87±3.65 mg GAE/100 mg FW), ferric reducing antioxidant power (FRAP) (11.87±3.49 - 72.47±3.07 mg TE/10g FW) and radical-scavenging activity (DPPH) (11.87±0.20 - 104.93±2.77 mg TE/100mg FW) compared with non-pigmented rice samples (3.76±0.88 - 11.24±1.39 mg GAE/100mg FW, 6.43±0.34 - 20.24±0.32 mg TE/10g and 2.76±0.44 - 14.76±1.00 mg TE/100mg respectively). The higher antioxidative properties were determined in darker pigmented rice such as black, red and brown rice samples compared to the non-pigmented rice samples. Pigmented rice samples also had the lowest minimum inhibitory concentrations (MIC) and minimum bacterial concentrations (MBC), where it demonstrated the higher antibacterial properties to reduce the growth of Bacillus cereus (ATCC® 11778™) compared to non-pigmented rice samples especially FPH (Black Floral glutinous) and THA (Thai Red). This study demonstrated that the darker pigmented rice and glutinous rice had the higher antioxidative and antibacterial activities.

Introduction

Rice (*Oryza sativa* L.) is an essential cereal crop for half of the humanity, especially for the people in the East and South-east Asian countries (Henderson *et al*., 2012; Phonsakhan and Kong-Ngern, 2015). FAO (2009) stated that in Malaysia, rice is one of the major agricultural crops and grown in 1.8 million hectares. The rice production systems have been classified into wetland or lowland and dryland or highland. The wetland contributes the highest total rice production in Malaysia compared to the dryland (Dano and Samonte, 2005). In Peninsular Malaysia, more than 90% of the rice cultivation areas are covered with wetland system, while in Sabah and Sarawak, dryland rice is mostly cultivated in many areas. Dryland rice either pigmented or non-pigmented is usually cultivated in a small scale or area and only for home consumption by rural or indigenous people (Sohrabi *et al.*, 2012). According to Fasahat (2012), although the white rice cultivars are generally consumed, pigmented genotypes have been traditionally consumed in East and South-east Asian countries such as Laos, Thailand, Cambodia, Vietnam, Malaysia, Indonesia, Myanmar, Bangladesh, India, China, Japan, Korea, Taiwan and the Philippines.

The pigmented rice is categorised base on the red, purple, black and brown colour on their bran layer. Anthocyanin is able to give the pigment colour to rice bran layer and the different bran layer among the rice genotypes are causes by genetic factor (Maekawa and Kita, 1984; Das *et al*., 2017). The rice bran has been reported to contain the highest antioxidative properties and beneficial effects against cancers such as liver, breast, leukimia, cervical and colorectal

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(Maekawa and Kita, 1984; Canan *et al*., 2012; Chen *et al.*, 2012; Henderson *et al.,* 2012). Rice bran also has higher antioxidative properties such as phenolic contents (TPC), tocochromanols and oryzanol especially in red and black rice compared to bright or white rice (Ryu *et al*., 1998; Ling *et al.,* 2001; Chen and Bergman, 2005; Zhang *et al*., 2006; Walter and Marchesan, 2011).

In previous studies, there is increasing interest in plant-derived substances i.e. rice extract as antibacterial agents to treat the disease. By using plant as ingredients in medicine, it is safer than synthetic or chemical compounds because it contains natural product. As an example, it is reported that rice extracts (Pumirat and Luplertlop, 2013) can inactivate the growth o*f Bacillus cereus* that causes vomiting and diarrheal syndrome. *B. cereus* is able to produce endospore cells and able to germinate into vegetative cells. It can produce emetic and diarrheal toxins (Ankolekar *et al.,* 2009; Ayari *et al*., 2016). Therefore, this study was undertaken to determine the physical, antioxidant and antibacterial properties of different indigenous rice (*Oryza sativa* L.) and glutinous rice (*Oryza sativa* var. glutinosa) varieties (crude extracts) from local cultivators or markets in the Peninsular of Malaysia. As for the antibacterial property of the rice extracts, only *B. cereus* was focused in this study, as it is the main pathogenic bacteria that contaminate rice. *Bacillus cereus* may cause vomiting and diarrheal syndrome and able to produce endospore cells and vegetative cells in pigmented and non-pigmented rice (Oh *et al*., 2012).

Materials and Methods

Rice samples and chemicals

Forty-two samples were used and collected from the local cultivators or markets of Peninsular Malaysia. They can be divided into two groups such as pigmented and non-pigmented rice and glutinous rice. The pigmented rice samples that were from UKM (Universiti Kebangsaan Malaysia) was RC9 (Red (UKMRC9), from five different supermarkets were PHC (Black (glutinous)), THA (Thai Red), FPH (Black: Floral (glutinous)), SBN (Sun Brown Jasmine) and MR7 (Brown Organic: MRQ-74). As for the non-pigmented rice samples from MARDI, Tanjung Karang, Selangor were MR9 (MR269) and MR3 (MR263) and from six different supermarkets were SMP (Sri Mutiara (glutinous)), PSS (Super Special Mosque), TAJ (Taj Mahal Herba Faiza), FPP (Floral (glutinous)), MUT (Naga Mutiara (fragrant)) and SU5 (Jasmine Super5).

All of the chemicals and reagents used were of analytical grade such as Folin–Ciocalteu phenol reagent: ferric chloride ($FeCl_3$•$6H_2O$) and HCl were obtained from Merck (Darmstadt, Germany). The DPPH (2,2-diphenyl-1-picrylhydrazyl), gallic acid, TPTZ (2,4,6-tris(2-pyridyl)-s-triazine), Trolox and sodium acetate trihydrate were purchased from Sigma (Missouri, USA). Sodium carbonate was purchased from Riedel-de Haën® (RDH) (Darmstadt, Germany), while glacial acetic acid was from Mallinckrodt Baker (Missouri, USA).

Rice samples preparation and extraction

Rice samples were pulverized into 0.5 mm size using rotor speed-mill (Fritsch Pulverisette 19, Sydney, Australia) and kept in airtight containers at chilled temperature (4°C). The method of Musa *et al*. (2011) was adopted with slight modification. The extraction procedure was conducted with 2 g of dried samples and 200 mL of 70% acetone (Merck, Germany), in order to achieve concentration of 10mg/ mL (Min *et al.* 2014; Tang *et al*., 2016). The extraction was done for 24 hr in room temperature. All the extraction samples were centrifuged using a centrifuge (Kubota, Japan) for 10 minutes at 14,800 rpm. The supernatants were collected for further analysis.

Colour of rice grain

Colour of rice grain was determined by using Minolta spectrophotometer CR-400 colorimeter (Minolta Co., Ltd., Osaka, Japan). Each rice sample was placed in petri dishes and the colour parameters (L^*: lightness, a^*:+ (redness) - (greenness), and b^*: + (yellowness) – (blueness)) were then read. Three replicates for each sample were determined (Yodmanee *et al*., 2015).

Measurement of total phenolic content (TPC)

The method of Slinkard and Singleton (1977) was adopted with slight modification. Rice extract (0.1 mL), gallic acid (standard calibration) and extracting solvent were placed in 2 mL deep well collection plates, followed by the addition of 400 µL of distilled water and 500 µL of diluted Folin–Ciocalteu reagent (10%; yellow colour). The mixtures were homogenized by pipetting and letting them to stand for 5 minutes, followed by the addition of 1 mL of 7.5% (w/v) of sodium carbonate [0.075 g of sodium carbonate (white crystals) mixed with 0.925 mL of distilled water]. The solutions (blue colour) were allowed to stand for 2 hr at room temperature and the absorbance was read at 765 nm wavelength by using spectrophotometer (SPECTROstarNano, Offenburg, Germany). The results were expressed

as mg of gallic acid equivalents per 100 mg of fresh sample (mg GAE/100 mg of FW). Three replicates for each sample were determined.

Determination of ferric reducing/ antioxidant power (FRAP)

The method of Benzie and Strain (1996) was adopted with slight modification. Fresh FRAP reagent was prepared by using 300 mM acetate buffer, pH 3.6 [3.1 g sodium acetate trihydrate, plus 16mL glacial acetic acid, made up to 1L with distilled water], 10 mM TPTZ (2,4,6-tri(2-pyridyl)-striazine) [0.031 g TPTZ] in 10 mL of 40 mM hydrochloric acid (HCl) [1.46 mL concentrated HCl made up to 1 L with distilled water] and 20 mM $FeCl_3$ $6H_2O$ [0.054 g of $FeCl_3$ $6H_2O$ mixed with 10ml of distilled water] in the ratio of 10:1:1 to give the working reagent. FRAP reagent (light brown colour) of 3,900 µL were freshly prepared and warmed at 37°C, was mixed with 100µL of the extracted sample, standards (Trolox), or extraction solvent (70% acetone) as blank reagent. After 30 minutes, the absorbance was read at 595 nm wavelength. The result was expressed as mg of Trolox equivalents per 10 g of fresh sample (mg TE/10 g of FW). All samples were done in three replications.

Determination of radical-scavenging activity (DPPH)

This assay was based on the method of Musa *et al*. (2011). Briefly, the decrease of the absorbance at 516 nm wavelength of the DPPH solution was measured by using spectrophotometer (SPECTROstarNano, Offenburg, Germany) after the addition of the blank or sample extract. An aliquot (3,900 µL) of methanolic DPPH solution (24 mg/L) (purple colour) was mixed with 100µL of extracted sample solution (10 mg/mL). The absorption was read at the beginning and after 30 minutes. The percentage of DPPH scavenging activity (%) was recorded and calculated using the following equation:

Radical scavenging activity = [Abs 516 nm (t = 0) - Abs516 nm (t = t´) x 10] /Abs 516 nm (t = 0).

The result was expressed as mg of Trolox equivalents per mg of fresh sample (mg TE/100mg of FW). All samples were done in triplicates.

Inoculum preparation

Bacillus cereus (ATCC® 11778TM) was chosen as the bacterial strain to determine the antibacterial property of the rice samples in this study. It is because *B. cereus* is a pathogenic bacterium that is associated with vomiting and diarrheal illness and frequently contaminates rice. To prepare the inoculum, the culture was streaked on Tryptic soy agar (TSA, Merck) plates and incubated at 30°C for 18 hr. Three to five well-isolated colonies of the same morphological type from the agar plates were selected. By using sterile collection swab, the top of each colony was touched and transferred to a test tube containing 5 mL of sterile distilled water. The density of the organism suspensions was adjusted to equal to the 0.5 McFarland standard (equivalent to 10^8 cfu/ mL) (Maregesi *et al*., 2013).

The minimal inhibitory concentration (MIC): broth macro-dilution

The minimal inhibitory concentration (MIC) was determined by using broth macro-dilution method, using final inocula of 10^5 cfu/ mL. Six different concentrations of rice extracts were prepared to obtain concentrations ranging from 3.125 to 100 mg/ mL and tested against *B. cereus* (ATCC® 11778TM). As refer to Rankin (2005), the inoculums suspension (10^8 cfu/ mL = 0.5 McFarland) were diluted by using three subsequent serial 1:10 dilutions to reach the final density of organism suspensions. One mL volumes of 10^5 cfu/ mL bacterial suspension were transferred into each test tube that contained each concentration of rice extract. The contents in the test tubes were mixed thoroughly and incubated at 37°C for 18hr. The MIC endpoint is the lowest concentration of rice extract at which there is no visible growth in the test tubes. All samples were done in triplicates and sterile distilled water was used as a negative control.

Minimal bactericidal concentration (MBC)

After MIC determination of the rice extract tested, an aliquot of 100 µL from all tubes in which no visible bacterial growth was observed were seeded in Mueller Hinton agar (MHA, Merck) plates. The plates were then incubated for 18hr at 37°C. The MBC endpoint is defined as the lowest concentration of antibacterial agent that kills >99.9% of the initial *B. cereus* (ATCC® 11778TM) population where no visible growth of the bacteria was observed on the MHA plates (Petrus *et al.,* 2011; Pumirat and Luplertlop, 2013).

Susceptibility test: disc diffusion

Rice extract was screened for antibacterial property using the standard paper disc diffusion assay as described by the Clinical and Laboratory Standards Institute (Ortez, 2005). The bacterial strain was streaked on MHA plates with sterile collection swab. Sterile filter paper discs (6 mm, Mastdiscs),

were loaded with 100 µL of 1 mg/ mL (w/v) rice extract. Sterile distilled water was used as negative control. The MHA plates were incubated at 37°C for 18 hr. Evidence of clear zone (including the disc diameter) was measured in millimeter (mm) unit. All rice samples were done in triplicates and the discs for each rice extracts were in duplicates.

Statistical analysis

All obtained data were done in triplicates and statistically analyzed by using SPSS program version 22 (SPSS Inc, Chicago, USA). Determination of comparison among the rice samples were determined by one-way ANOVA followed by Duncan's multiple Range tests at the significance level of $p<0.05$.

Results and Discussion

Colour of rice grain

Table 1 shows the colour parameters (L^*, a^* and b^*) of the ten varieties of rice and four varieties of glutinous rice grains. L^* values, which expresses the lightness, were in the range of 40.95-107.97. The values of a^* (+ redness; - greeness) and b^* (+ yellowness; - blueness) were in the range of -12.11-13.72 and -3.26-16.16 respectively. The L^* values of non-pigmented rice samples (MR9, MR3, SMP, PSS, TAJ, FPP, MUT and SU5) were significatly higher than those of the red, black and brown rice samples at $p<0.05$. This results indicated that the non-pigmented rice samples had higher lightness than the red, black and brown rice samples. The a^* values for all pigmented rice samples (RC9, PHC, THA, MR7, FPH and SBN) were significantly higher than the non-pigmented rice samples at $p<0.05$. As for the the b^* values in pigmented samples (RC9, THA, MR7 and SBN), they varied more than those in the non-pigmented rice samples, except for PHC (Black glutinous) and FPH (Black glutinous) that had lower b^* values compared to the non-pigmented rice samples. According to Escribano- Bailón *et al.* (2004), Yawadio *et al*. (2007) and Yodmanee *et al.* (2011), the differences in grain colour could depend on the form of anthocyanins and rice genotypes. The pigment generally found in pigmented rice and glutinous rice played an important role in reducing the risk of cancer and other chronic diseases. It is because of their free radicals scavenging capacities.

Antioxidant properties

The TPC (Total phenolic content), FRAP (Ferric Reducing/ Antioxidant Power) and DPPH (Radical-Scavenging Activity) of rice and glutinous rice extracts were shown in Table 1. The phenolic compounds may directly contribute to antioxidant properties of rice; therefore, TPC in rice and glutinous rice grains were measured. The TPC was expressed as miligrams of gallic acid equivalents (FW) per 100 miligrams of samples. TPC ranged from 3.76 to 104.87 mg GAE/100 mg extract. Black glutinous rice (FPH, and PHC) and red rice (THA and RC9) had significantly highest TPC compared to brown rice (MR7 and SBN) samples and non-pigmented rice samples at $p<0.05$. Moreover, FPH black glutinous rice extract contained the highest TPC (104.87±3.65 mg GAE/100mg extract) which was 9 times greater than SBN (Sun Brown Jasmine) brown rice (11.12±2.49 mg GAE/100mg extract) and 28 times higher than SMP (Sri Mutiara) white glutinous rice (3.76±0.88 mg GAE/100mg extract). It was similar to the previous studies that pigmented rice like black, red and brown rice tend to have higher TPC than non-pigmented rice (Ratanachithawat *et al.*, 2010; Chen *et al.*, 2012; Jun *et al*., 2012; Thitipramote *et al*., 2016). Zhang *et al.* (2015) also showed that the TPC of black rice was significantly higher than the other pigmented rice and white rice, which was also confirmed by this study. Similarly, Muntana and Prasong (2010) found that the red rice had the highest TPC than the black rice and white rice. They also showed that the Thai Red rice samples contained TPC (122.39 mg GAE/100mg extract) that was higher than in this study (92.62 mg GAE/100mg extract).

For DPPH radical scavenging activity ranged from 6.43-72.47 mg GAE/100mg FW extract. For pigmented rice samples, the DPPH scavenging assay were ranged from 11.87-72.47 mg GAE/100mg FW extract and the highest DPPH scavenging assay was FPH black glutinous rice (72.47±3.07 mg GAE/100mg FW extract). FPH had 6 times higher DPPH scavenging assay than SBN (Sun Brown Jasmine) brown rice (11.87±3.4 mg GAE/100mg FW extract). Among the non-pigmented rice samples (MR9, MR3, SMP, PSS, TAJ, FPP, MUT and SU5), TAJ (Taj Mahal Herba Faiza) white rice had the highest DPPH scavenging assay (20.24±0.32 mg GAE/100mg FW extract) and 3 times higher than SMP (Sri Mutiara) white glutinous rice (6.43±0.34 mg GAE/100mg FW extract). Anggraini *et al*. (2015) had shown that there is correlation of colour parameters with the antioxidant property of pigmented rice, i.e. the darker the rice, the higher antioxidant properties such as DPPH scavenging assay than the brightness of rice especially in white rice.

The FRAP (Ferric Reducing/ Antioxidant Power) assay in pigmented rice samples had higher range (11.87-104.93 mg TE/10g FW extract) than the non-

Table 1. Colour determination, total phenolic content (TPC), DPPH scavenging assay and Ferric reducing antioxidant power (FRAP) assay of rice and glutinous rice samples

Samples	Code	Colour parameters			Antioxidant activities		
		L* lightness	a* (+) redness, (-) greenness	b* (+) yellowness, (-) blueness	TPC (mg GAE/ 100mg FW)	% DPPH (mg TE/ 100mg FW)	FRAP (mg TE/ 10g FW)
Red (UKMRC9)	RC9	56.08±0.66^{h}	8.36±0.70^{b}	16.16±1.27^{a}	76.80±2.06^{c}	44.04±2.51^{b}	79.68±1.17^{c}
Black (glutinous)	PHC	40.95±0.55^{j}	3.97±0.35^{c}	-3.26±0.63^{j}	72.70±2.28^{d}	47.570±0.50^{c}	85.65±0.77^{b}
Thai Red	THA	45.61±0.19^{j}	13.72±1.10^{a}	10.25±1.17^{d}	92.62±1.59^{b}	62.82±0.60^{b}	85.93±2.18^{b}
Brown Organic (MRQ-74)	MR7	84.50±3.03^{a}	-3.85±0.24^{d}	15.50±1.54^{b}	15.38±1.34^{e}	16.74±1.18^{ef}	11.87±0.20^{e}
Black: Floral (glutinous)	FPH	46.34±0.63^{i}	3.36±0.12^{c}	0.68±0.25^{h}	104.87±3.65^{a}	72.47±3.07^{a}	104.93±2.77^{a}
Sun Brown Jasmine	SBN	87.00±1.08^{f}	-7.07±0.55^{e}	14.14±1.17^{bc}	11.12±2.49^{ef}	11.87±3.49^{g}	15.13±3.66^{d}
MR269	MR9	90.97±0.80^{cd}	-7.70±0.26^{e}	6.03±0.59^{f}	11.24±1.39^{ef}	12.49±1.77^{g}	8.09±0.47^{f}
MR263	MR3	96.48±0.83^{c}	-8.54±0.17^{f}	7.07±0.30^{ef}	10.83±2.58^{f}	11.58±1.72^{g}	5.94±2.92^{fg}
Sri Mutiara (glutinous)	SMP	104.82±2.09^{a}	-10.83±0.32^{h}	8.09±0.74^{e}	3.76±0.88^{h}	6.43±0.34^{h}	2.76±0.44^{h}
Super Special Mosque	PSS	92.25±1.19^{d}	-7.43±0.33^{e}	5.91±1.18^{f}	8.80±0.86^{fg}	13.85±1.86^{fg}	7.26±0.61^{fg}
Taj Mahal Herba Faiza	TAJ	89.73±0.67^{e}	-9.94±0.26^{g}	13.55±0.66^{c}	15.30±2.78^{e}	20.24±0.32^{e}	14.76±1.00^{d}
Floral (glutinous)	FPP	107.97±1.22^{a}	-12.11±0.32^{i}	10.76±1.19^{d}	6.82±3.74^{fgh}	18.15±3.60^{e}	4.49±0.87^{gh}
Naga Mutiara (fragrant)	MUT	87.61±0.38^{f}	-6.92±0.15^{e}	4.04±0.40^{g}	8.07±2.07^{fg}	17.08±1.07^{ef}	6.75±1.00^{fg}
Jasmine Super5	SU5	90.84±0.50^{cd}	-9.51±0.20^{g}	6.14±0.50^{f}	6.44±2.96^{gh}	16.95±1.26^{ef}	4.79±1.42^{gh}

$^{a-h}$: Different alphabet at same column show significant different at ($p<0.05$);
± : standard deviation at min (colour parameters and antioxidant activities) for 3 replication test (n=3)

pigmented rice samples (2.76-14.76 mg TE/10g FW extract). The FPH black glutinous rice (104.93±2.77 mg TE/10g FW extract) had 9 times higher FRAP than the MRQ-74 organic brown rice (11.87±0.20 mg TE/10g FW extract) and 38 times higher than the SMP (Sri Mutiara) white glutinous rice extract (2.76±0.44 mg TE/10g FW extract). FPH also had the highest FRAP assay than all the fourteen varieties of rice samples. As expected, the pigmented rice like black rice, red rice and brown rice had higher antioxidant properties as determined by FRAP assay (Chunthaburee *et al.,* 2015). TAJ (Taj Mahal Herba Faiza) white rice (14.76±1.00 mg GAE/100mg extract) had higher FRAP assay than the other non-pigmented rice samples. It also had 5 times higher FRAP assay than SMP (Sri Mutiara) white glutinous rice. SMP had the lowest FRAP assay with only 2.76±0.44mg TE/10g FW compared to all the rice samples in this study. The correlation between colour parameter (L^*, a^* and b^*) and antioxidant properties (TPC, DPPH and FRAP) were shown in Table 2. Generally, the correlation between L^* and antioxidant properties for TPC, DPPH and FRAP were lowest compared to other colour parameter. It showed that the higher the lightness *(L^*)* value of the samples, the lower the value of antioxidant properties (Dutta *et al.*, 2012). On the other hand, the correlation between antioxidant properties (TPC, DPPH and FRAP) with antibacterial properties (MIC and MBC) were lower (negative value) than the inhibition zone (Table 3). It showed that the higher the antioxidant properties, the lower the concentration of MIC and MBC.

Antibacterial properties of rice extracts

Determination of the potential antibacterial properties of rice and glutinous rice samples against pathogenic bacteria-causing diarrhea and vomiting in human bodies was done in this study. The antibacterial effects of forty-two rice samples extracts on the control strain of *B. cereus* (ATCC® 11778TM) were shown in Table 3 and Figure 1. As referred to Table 3, the average MICs of the pigmented rice (RC9, PHC, THA, MR7, FPH and SBN) samples' extracts against *B. cereus* were 11.46 mg, which were significantly

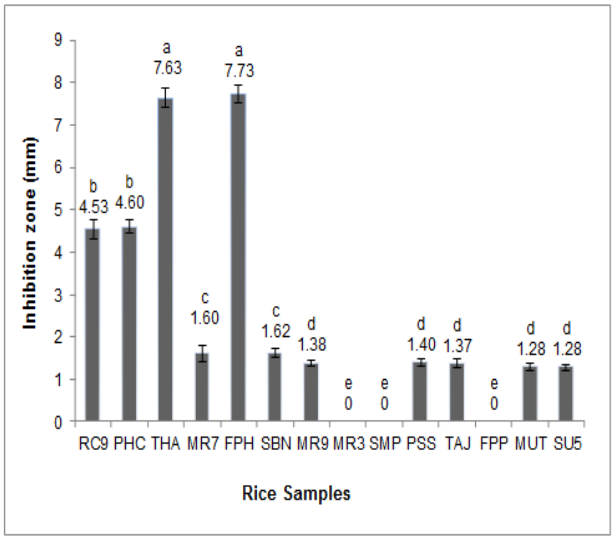
Table 2. Pearson's correlation coefficients of antioxidant properties with antibacterial properties and colour parameter of pigmented and non-pigmented rice samples

Correlation coefficient (r)	L*	a*	b*	TPC	% DPPH	FRAP
L*				-0.95	-0.92	-0.96
a*				0.92	0.87	0.90
b*				-0.24	-0.29	-0.28
TPC						
% DPPH						
FRAP						
MIC	0.75	-0.67	0.06	-0.64	-0.61	-0.66
MBC	0.93	-0.85	0.23	-0.83	-0.81	-0.84
Inhibition zone	-0.94	0.91	-0.24	0.97	0.97	0.95

($p<0.05$) lower than the non-pigmented (MR9, MR3, SMP, PSS, TAJ, FPP, MUT and SU5) rice samples at an average of 53.13 mg. Among the rice samples, THA red rice (Thai Red) and FPH black glutinous rice (Floral) had MICs at the lowest concentration of 3.125 mg. Both of the rice samples also had MICs 2 times lower than RC9 red rice (UKMRC9) and PHC black glutinous rice, 8 times lower than MR7 brown organic rice (MRQ-74), SBN brown rice (Sun Brown Jasmine), PSS white rice (Super Special Mosque), TAJ white rice (Taj Mahal Herba Faiza) and SU5 white rice (Jasmine Super5), 16 lower than MR9 white rice (MR269), SMP white glutinous rice (Sri Mutiara) and MUT white rice (Naga Mutiara (fragrant)), and 32 times lower than MR3 white rice (MR263) and FPP white glutinous rice (Floral).

The MBCs for pigmented rice samples also showed the lowest average concentration at only 22.92 mg which was 3 times lower than the non-pigmented rice samples (64.29 mg). The lowest MBCs among all samples were THA red rice (Thai Red) and FPH black glutinous rice (Floral) at 6.25 mg and >16 times lowest than MR3 white rice (MR263). It showed that the pigmented rice samples with highest antioxidative properties were able to reduce the growth and prevalence of *B. cereus* in rice and glutinous rice at low concentration.

Figure 1 showed the susceptibility test results by using disc diffusion and measuring the zone of inhibition on MHA agar. At concentration of 1 mg, the pigmented rice samples were able to reduce B. cereus at a range of 1.60 - 7.73 mm compared to the non-pigmented rice samples for only at 0 - 1.40mm. FPH black glutinous rice had the highest zone of inhibition at 7.73 mm and based from the antioxidative properties (TPC, DPPH and FRAP), it also showed the highest concentration compared to the other rice samples. This demonstrated the fact that the higher the antioxidative properties of the rice samples, the stronger were they against bacterial growth (antibacterial properties). The results were supported by previous studies of Chakuton *et al.* (2012), Kim *et al.* (2012), Deng *et al*. (2013), Gonzalez *et al*. (2013) and Pumirat and Luplertlop (2013), where pigmented rice (*Oryza sativa* L.) was able to be a potential natural antibacterial agent. The



[a-e]: Different alphabet at rice samples show significant different at ($p<0.05$);
Error bar : standard deviation at min (inhibition zone) for 3 replication test (n=3)
RC9 = Red (UKMRC9); PHC = Black (glutinous); THA = Thai Red; MR7 = Brown Organic (MRQ-74); FPH = Black: Floral (glutinous); SBN = Sun Brown Jasmine; MR9 = MR269; MR3 = MR263; SMP = Sri Mutiara (glutinous); PSS = Super Special Mosque; TAJ = Taj Mahal Herba Faiza; FPP = Floral (glutinous); MUT = Naga Mutiara (fragrant); SU5 = Jasmine Super5.
Figure 1. Inhibition zone (mm) for raw rice samples against control strain of *Bacillus cereus* (ATCC® 11778™)

Table 3. Minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) breakpoints for pigmented and non- pigmented rice samples against control strain of *Bacillus cereus* (ATCC® 11778™)

Sample	Code	MIC (mg/ mL)	MBC (mg/ mL)
Red (UKMRC9)	RC9	6.25	12.50
Black (glutinous)	PHC	6.25	12.50
Thai Red	THA	3.125	6.25
Brown Organic(MRQ-74)	MR7	25.00	50.00
Black: *Floral* (glutinous)	FPH	3.125	6.25
Sun Brown Jasmine	SBN	25.00	50.00
MR269	MR9	50.00	50.00
MR263	MR3	100.00	>100.00
Sri Mutiara (glutinous)	SMP	50.00	100.00
Super Special Mosque	PSS	25.00	50.00
Taj Mahal Herba Faiza	TAJ	25.00	50.00
Floral (glutinous)	FPP	100.00	100.00
Naga Mutiara (fragrant)	MUT	50.00	50.00
Jasmine Super5	SU5	25.00	50.00

pigmented rice like black, red and brown contain a variety of bioactive constituents such as amino acids, anthocyanins, essential oils, flavones, phenolics, tannin, tocols, sterols, and γ-oryzanols (Chakuton *et al*.,2012, Kim *et al*., 2012, Deng *et al*., 2013, Gonzalez *et al*., 2013, Pumirat and Luplertlop, 2013). A study by Huang *et al.* (2012) has shown that glutinous rice was able to reduce the growth of pathogenic bacteria like *B. cereus* and treat stomach upset symptom such as diarrhoea and nausea.

The correlation (Table 2) between antibacterial properties (MIC, MBC and inhibition zone) with colour parameters and antioxidant properties showed that a^* for both MIC and MBC was the lowest (negative value) compared to the Inhibition zone. It showed that the pigmented rice had higher antioxidant properties and able to inhibit the growth of *B. cereus* with lower MIC and MBC concentrations.

Conclusions

Rice and glutinous rice contain pigments which are one of the good sources of antioxidative compounds, including phenolic. As far as antioxidant and their activities are concern, it was found that pigmented rice samples contained higher antioxidative properties than the non-pigmented samples. In addition, the darker pigmented rice has the highest antioxidative properties compared to brighter rice. However, if compared with the colour, the black rice (FPH; glutinous) had higher antioxidative properties such as TPC, DPPH and FRAP than the red and brown rice. FPH black glutinous rice and THA red rice both showed the lowest MICs and MBCs compared to all other rice samples. It demonstrated that the pigmented rice samples were able to reduce the growth and prevalence of *B. cereus* (ATCC® 11778TM) much better than the non-pigmented rice.

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