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Anthocyanin content in the black scented rice (*Chakhao*): its impact on human health and plant defense

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Abstract The major anthocyanin compositions of the two black scented rice cultivars (*Chakhao Poireiton and Chakhao Amubi*) were studied using HPLC. Four main anthocyanins, i.e., delphinidin 3-galactoside, delphinidin 3-arabinoside, cyanidin 3-galactoside and cyanidin 3-glucoside were identified in *Chakhao Poireiton* while three main anthocyanins, delphinidin 3-galactoside, delphinidin 3-arabinoside and cyanidin 3-galactoside were identified in *Chakhao Amubi*. In both the cultivars, delphinidin 3-galactoside is the most predominant anthocyanin. The total monomeric anthocyanin content and total phenolics were measured using a modified pH differential method and modified Folin-Ciocalteu method, respectively. The total anthocyanin content in *Chakhao Poireiton* was found to be 740 mg/kg and *Chakhao Amubi* was 692 mg cyanidin 3-glucoside/kg of dried powder sample. And the total phenolic content was 577 and 500 mg/100 g of the dried powder sample as Gallic acid equivalent in *Chakhao Poireiton and Chakhao Amubi*, respectively. The anthocyanin extract showed strong antioxidant activity by DPPH assay, the highest scavenging activity of *Chakhao Poireiton and Chakhao Amubi were* 70.28 % and 69.73 %, respectively. From the study it can be suggested that supplementation of the black scented rice in the diet will have a great impact on human health. The rich anthocyanin and phenolic help to protect the plant from rice diseases and pests.

Keywords Black scented rice · Anthocyanin · HPLC · Phenolics content · Plant defense

1 Introduction

Rice is one of the most economically important cereal crops in the world and a significant staple food for feeding much of the world's population. White rice is the most commonly consumed rice, but there are several rice cultivars which contain color pigments, such as black and red rice. The dark purple color of Black rice is due to the high anthocyanin content, located in the pericarp layers (Takashi et al. 2001). Rice varieties with colored pericarp (other than white and red) are usually known as "black rice". In Asian countries, Black rice is popular and mixed with white rice prior to cooking to enhance the flavor, color and nutritional value (Yang et al. 2003).

Anthocyanins are a group of reddish-purple water-soluble flavonoids, which are the primary pigments in the red and black grains and give the attractive red, purple and blue colors of many flowers, fruits, and vegetables. It is estimated that more than 400 naturally occurring anthocyanins have been found (Kong et al. 2003). Early studies showed that polyphenols such as plant anthocyanins are helpful for cardiovascular health (Stoclet et al. 2004; Manach et al. 2005). The anthocyanin extract from black rice enhances plaque stability in an ApoE-deficient mouse model (Xia et al. 2006). In rabbits, supplementation of black rice in the diet, improved the lipid profile and increased glutathione peroxidase activity (Ling et al. 2002). Supplementation of black rice pigment fraction to the diet significantly inhibited rabbits atherosclerotic plaque formation and in apolipoprotein (APO) E-deficient mice (Ling et al. 2002; Xia et al. 2003).

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Anthocyanins have also been observed to function in a diverse array of plant/animal interactions, which include the attraction of pollinators and frugivores and the repellence of herbivores and parasites (Lev-Yadun and Gould 2009). Plant secondary metabolites include phenol, phenolic acids, quinines, flavones, flavonoids, flavonols, tannins and coumarins and all these compounds have plant defensive mechanisms against pathogenic microorganisms. It has been reported that flavonoids and flavonoid derivatives play important roles in the development of the plant, in protection against UV radiation, attraction of insects for pollination and plant defense responses (Harborne and Williams 2000; Winkel-Shirley 1996). Previous studies reported that the plant extracts are being used as phytochemicals to protect against several plant diseases like bacterial blight, stem rot, brown rot, root rot and brown spot due to their rich phenolic compounds (Enyiukwu et al. 2014).

The black scented rice (*Chakhao*) of Manipur, a North-eastern State of India has their importance as scented and are dark purple color which is used for the community feast as well as ceremonial purposes as a delicacy. These are one of the high rated dishes serve as desserts, flakes, bread, cakes, beverages and a special snack "Utong Chak" prepared within bamboo sticks. The black scented rice (*Chakhao*) of Manipur has been used by the traditional medical practitioners also. They are sold in the local markets at a premium rate. The black scented rice cultivars of Manipur are poor yielders which are found only in this state of India and little is known about it throughout the Indian regions. There is a huge demand in the domestic market, having possibilities for export, but the farmers of Manipur are neglecting to cultivate these cultivars as they are low yielding. Thus, the present study was taken up to show that the black scented rice of Manipur, India may have certain health benefits when added to the diet. Not only this, understanding and considering their importance the farmers may continue raising black scented rice and may include these cultivars in the crop improvement program. The study may let understand the public its importance. Recently, there has been only a few studies on the black scented rice of Manipur regarding the germplasms collection, conservation, genetic diversity (Singh and Sharma 1998; Roy et al. 2014) and Das et al. 2014, reviewed on its potential values. To the best of our knowledge, this is the first and foremost study considering the anthocyanin compositions of the black scented rice of Manipur, India. The present study was taken up to find out the anthocyanin content, phenolics content, antioxidant activity and the composition of anthocyanin in two black scented rice cultivars (*Chakhao Poireiton and Chakhao Amubi*). Thus, the current study may develop a better understanding of the exploration of the black scented rice of Manipur and the improvement of these cultivars.

2 Material and methods

2.1 Two black scented rice cultivars

(*Chakhao Poireiton and Chakhao Amubi*) were kindly provided by the Department of Plant Breeding and Genetics, Central Agricultural University (Imphal, India) and were used in the study. The samples were ground using a grinder. The analysis was conducted on the ground samples.

2.2 Chemicals

The solvents: methanol, acetonitrile and phosphoric acid were HPLC grade (Sigma, USA). The standard cyanidin 3- O glucoside was purchased from Sigma, USA. 5 g of sample powder was extracted with 300 ml of acidified methanol (1 N HCl, 85:15, *v/v*) (Kim et al. 2008) using Soxhlet (apparatus) for 16 h at 60 °C (Gholivand and Piryaei 2014; Huang et al. 2009). Changing temperature between 25 and 60 °C during extraction of anthocyanin and storage of the anthocyanin extracts at 4 °C does not seem to have a significant effect on the absorbance readings (Shipp and Abdel-Aal 2010). Increasing temperature from 25 to 60 °C during anthocyanin extraction from purple and blue wheat increased the absorbance readings by 15 % (Shipp and Abdel-Aal 2010). The extracted extract was evaporated/ concentrated using a rotatory evaporator to dryness and reconstituted in acidified methanol (5 ml). The reconstituted extract was filtered through a 0.45 mm (45 micron) syringe filter prior to HPLC analysis. The standards were dissolved in acidified MeOH (1 N HCl, 85:15, v/v) to obtain the concentrations of 1 mg/ml.

2.3 Extraction and purification

The HPLC method for anthocyanin measurement was made based on methods reported earlier with some modifications (Mazza et al. 2004; Naczk and Shahidi. 2006; Jing et al. 2007; Jia et al. 2008; Wang 2014). Chromatographic analysis was performed on the Thermo Spectra System, HPLC System (Thermo Scientific, USA) equipped with an (autosampler/injector end) UV–VIS 3000 (AS 3500 detector) HPLC P-4000 pump, SN 4000 controller and chromleon 4.2 software. The separation of anthocyanins was done by C18 column (250× 4.6 mm). Elution was performed using mobile phase A (96 % buffer solution. Buffer solution is 20 mM Sodium dihydrogen phosphate adjusted to pH 2.5 by the addition of phosphoric acid) and mobile phase B (4 % acetonitrile). The gradient condition was as follows:- 0–10 min, 15%B, 10–20 min, 25 B, 20–30 35 and 30–40 45 %. The flow rate was 1 ml/min and elution of compounds of interest was monitored at wavelength 517 nm.

2.4 Total phenolics

Total phenolics were measured using a modified Folin - Ciocalteu method (Waterhouse 2001; Jing et al. 2007). Briefly, a series of tubes was prepared with 15 ml of water and 1 ml of Folin—Ciocalteu reagent. Then, 1 ml of samples, Gallic acid dilutions (standards) and water blank was added into tubes, mixed well, and left to stand at room temperature for 10 min. The 20 % Na2CO3 solution (3 ml) was added to each test tube and mixed well before they were put in a dry-bath incubator at 40 °C for 20 min. After incubation, tubes were immediately cooled down in an ice bath. The absorbance of samples and standards was measured at 755 nm using a Cecil UV–visible spectrophotometer. Total phenolics were calculated as Gallic acid equivalents based on a Gallic acid standard curve.

2.5 Total monomeric anthocyanin was measured using the pH differential method

The total monomeric anthocyanin content was measured according to the methods described earlier with some modifications (Giusti and Wrolstad 2001; Jing et al. 2007; Hosseinian et al. 2008). Two dilutions of the samples were prepared, one for pH 1.0 using 0.025 M potassium chloride buffer and the other for pH 4.5 using 0.4 M sodium acetate buffer. The samples were diluted 10 times to a final volume of 2 ml. The absorbance of each sample was measured at 520 nm against distilled water as blank. The concentration (mg/L) of each sample was calculated according to the following formula and expressed as cyanindin-3- glucoside (Cy-3-glc) equivalents:

$$\frac{A\ \mathrm{X\ MW\ X\ DF\ X}\ 10^{3}}{€\,\mathrm{X}\,1}$$

Where A is the absorbance=$(A_{\lambda\text{vis-max}})_{\text{pH 1.0}}$ - $(A_{\lambda\text{vis-max}})_{\text{pH 4.5}}$, MW is the molecular weight=449.2 g/mol for Cy-3-glc, DF is the dilution factor (0.2 ml sample is diluted to 2 ml, DF=10), and € is the extinction coefficient (L x cm^{-1} x mol^{-1})=26,900 for Cy-3-glc, where L (pathlenght in cm) =1.

2.6 DPPH radical scavenging activity

The determination of the quenching of free radical activity was done using 2,2 -diphenyl-1-picryl hydrazyl (DPPH) described by Devi et al. 2015, with some modifications. One ml of 0.1 mM solution of DPPH free radical in methanol was mixed with 1 ml of the extract (50, 100, 150 μg/ml) and after mixing the solutions were incubated for 30 min and then absorbance was measured at 517 nm. Similarly 1 ml of methanolic solution of ascorbic acid (50, 100, 150 μg/ml) were mixed with 1 ml of DPPH methanolic solution and absorbent were recorded at the same wavelength. The radical scavenging activity was calculated using the following formula:

$$\text{DPPH free radical scavenging activity}(\%) = [(\mathrm{A0}-\mathrm{A1}/\mathrm{A0}) \times 100]$$

A0 = the absorbance of the control
A1 = the absorbance in the presence of anthocyanin extracts or standards.

2.7 Statistical analysis

Samples were analysed in triplicate & one way analysis of variances performed using SAS 9.1 significant differences were detected at $P<0.05$.

3 Result

Organic solvents such as methanol and acetone are usually used for extraction of anthocyanins, the acidified MeOH showed the highest extraction efficiency (70–100 %) (Naczk and Shahidi 2006; Mazza et al. 2004). Extraction of anthocyanins is conducted with methanol or ethanol containing a small amount of acid (15 % 1.0 mol/L HCL) with the objective of obtaining the flavylium cation form, which is stable in a highly acid medium (Abdel-Aal and Hucl 1999).

In the present study, anthocyanins were extracted from black scented rice using acidified methanol (Soxhlet). The total anthocyanin content in *Chakhao Poireiton* was found to be 740 mg/kg and *Chakhao Amubi* was 692 mg cyanidin 3-glucoside/kg of powdered rice. And the total phenolic content was 577 and 500 mg/100 g of the powdered sample as Gallic acid equivalents in *Chakhao Poireiton and Chakhao Amubi*, respectively. In the DPPH free radical scavenging assay, anthocyanin extract of *Chakhao Poireiton* exhibited 42.91 % scavenging activity at 50 μg/ml, 55.20 % scavenging activity at100 μg/ml, and 70.28 % scavenging activity at 150 μg/ml, respectively. And in the DPPH free radical scavenging assay, anthocyanin extract of *Chakhao Amubi* exhibited 39.35 % scavenging activity at 50 μg/ml, 53.86 % scavenging activity at100 μg/ml, and 69.73 % scavenging activity at 150 μg/ml, respectively. The standard ascorbic acid showed 46.06, 89.03 and 93.73 % scavenging activity at 50, 100 and 150 μg/ml, respectively. We also analyzed anthocyanin extract by HPLC method using the gradient system. Anthocyanins were identified according to the HPLC retention time by comparison with authentic standards and published data (Jing et al. 2007; Hosseinian et al. 2008; Jia et al. 2008; Lee et al. 2009). Figures 1 and 2 show the HPLC chromatogram of *Chakhao Poireiton* and *Chakhao Amubi*, respectively. The total anthocyanins reported, includes both identified and non-identified HPLC peaks (Figs. 1 & 2: Table 1). Due to lack of corresponding anthocyanin standards and published data corresponding to the peak, some of the peaks

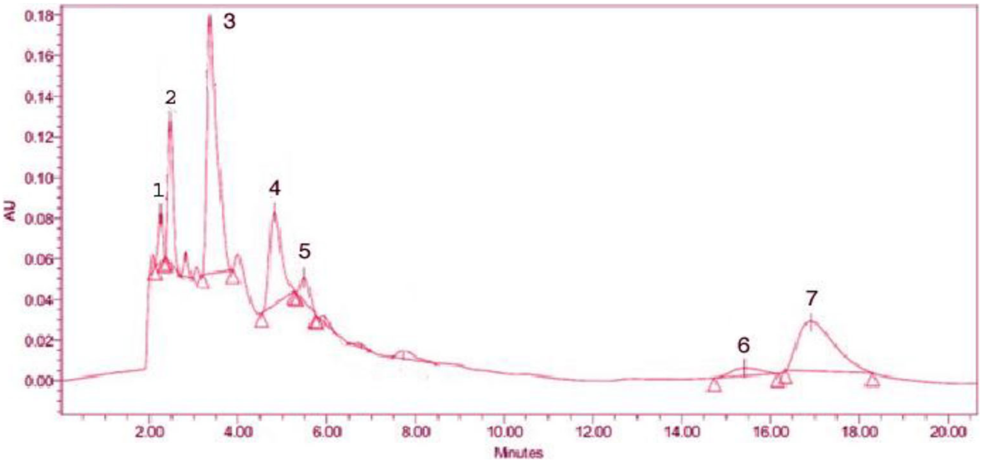

Fig. 1 HPLC chromatogram (517 nm) for anthocyanin distribution in *Chakhao Poireiton*

remain unlabelled. By comparing with the previously reported data (Jing et al. 2007; Hosseinian et al. 2008; Jia et al. 2008; Lee et al. 2009), four main anthocyanins, i.e., delphinidin 3-galactoside (Dp-3-gal), delphinidin 3-arabinoside (Dp 3-ara), cyanidin 3-galactoside (Cy-3-gal) and cyanidin 3-glucoside (Cy-3-glc) were identified in *Chakhao Poireiton* (Fig. 1: Table 1). Three main anthocyanins, delphinidin 3-galactoside (Dp-3-gal), delphinidin 3-arabinoside (Dp-3-ara) and cyanidin 3-galactoside (Cy-3-gal) were identified in *Chakhao Amubi* (Fig. 2: Table 1). In both the samples, Dp-3-gal was found to be the most predominant anthocyanin.

4 Discussion

4.1 Peak identification and assignment

Interest in the anthocyanin content of colored cereals such as purple wheat has increased because of their potential as nutraceutical ingredients and functional foods (Choia et al. 2007). As such, there is also an increased in the interest of supplementing colored rice in the diet. Anthocyanins are also regarded as safe and effective food colorants (Manach et al. 2004). Identification and peak assignment of anthocyanins in all foods were based on comparison of their retention times (RT) with those of standards and published data (Jing et al. 2007; Hosseinian et al. 2008; Jia et al. 2008; Lee et al. 2009). Four main anthocyanins were identified in *Chakhao Poireiton* (Fig. 1: Table 1) i.e., peak no. 3 as Dp-3-gal (RTmins. ranging from 3 to 3.9), peak no. 5 as Dp 3-ara (RTmins. ranging from 5.2 to 5.7), peak no. 6 as Cy-3-gal (RTmins. ranging from 14.6 to 16.2) and peak no. 7 as Cy-3-glc (RTmins. ranging from 16.4 to 18.4). Three main anthocyanins, peak no. 2 as Dp-3-gal (RTmins. ranging from 3.0 to 3.9), peak no. 3 as Dp 3-ara (RTmins. ranging from 5.2 to 5.7) and peak no. 4 as Cy-3-gal (RTmins. ranging from 14.6 to 16.2) were identified in *Chakhao Amubi* (Fig. 2: Table 1). There were also some unidentified peaks (Figs. 1 & 2: Table 1) observed in both the black scented rice cultivars that showed UV absorption at 517 nm at shorter retention time. This is due to the lack of standards and published data. The study on purple corncob anthocyanins showed that the anthocyanins could be acylated accounting for 35.6–56.0 % of total anthocyanins (Jing et al. 2007). In the study, Dp 3-glc showed the shortest RT (Table 1). Whereas cyanidin showed the longest RT i.e., Cy-3-glc and Cy-3-gal (Table 1) in *Chakhao Poireiton* and *Chakhao Amubi*, respectively. Cy-3-glc and peonidin 3- glucoside (Pn-3-glc) are the two major anthocyanins present in the Korean black rice (*Heugjinjubjeo*) (Park et al. 2008). Lee 2010, also showed Cy-3-glc exhibited a markedly higher content in the black rice. Dp-3-gal, Dp-3-ara, Cy-3-ara, Pt (Petunidin)-3-glc, Cy-3-gal, Cy-3-glc, Pg (pelargonidin)-3-

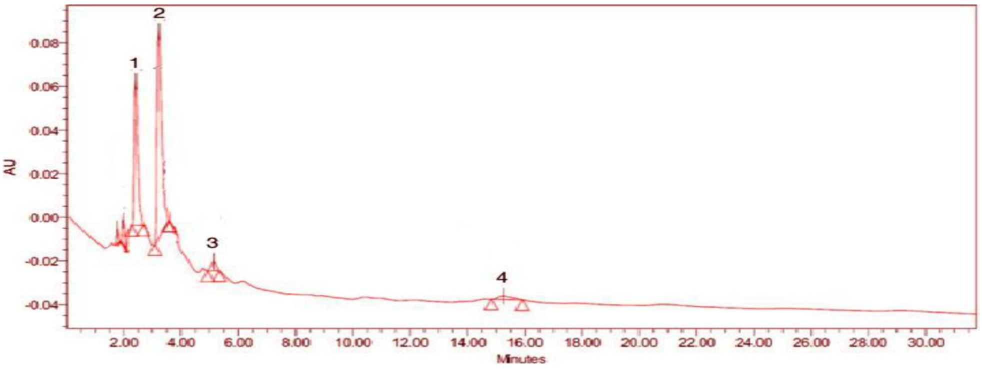

Fig. 2 HPLC chromatogram (517 nm) for anthocyanin distribution in *Chakhao Amubi*

Table 1 Chromatographic characteristics of anthocyanins from *Chakhao Poireiton* and *Chakhao Amubi*

Peak no.	RT (min)	Area (%) (517 nm)	Compound
Chakhao Poireiton			
1	2.2–2.4	2.9	unidentified
2	2.4–2.7	10.4	unidentified
3	3.0–3.9	37.5	Delphinidin 3-galactoside
4	4.6–5.2	16.1	unidentified
5	5.2–5.7	3.1	Delphinidin 3-arabinoside
6	14.6–16.2	3.4	Cyanidin 3-galactoside
7	16.4–18.4	26.7	Cyanidin 3-glucoside
Chakhao Amubi			
1	2.2–2.5	32.0	unidentified
2	3.0–3.9	62.1	Delphinidin 3-galactoside
3	5.2–5.7	3.0	Delphinidin 3-arabinoside
4	14.6–16.2	2.9	Cyanidin 3-galactoside

glc, Pn-3-glc, Mv (malvidin)-3-glc, Cy-cl (chloride), Pg-3-gal, Pg-3-ara and Pn-3-ara were identified in purple wheat (Hosseinian et al. 2008). Cy 3- glc, Pg 3-glc, Pn 3-glc, Cy-3-(malonyl)glc, Cy 3-(malonyl)glc, Pg 3-(malonyl)glc, Pn 3-(malonyl)glc and Cy-3- dimalonylglc were the major anthocyanins identified in the purple corncob (Jing et al. 2007). Two main anthocynains, i.e., Cy 3-rut (rutinoside) and Cy 3-glu were identified in mulberry (*M. rubra*) and four main anthocyanins, Pt (petunidin) 3-rut, Dp 3-glu, Dp 3-rut and Mv 3-rut were identified in *Liriope platyphylla* fruits (Wang 2014). Six main anthocyanins, including Pn-3,5-glc, Pn-3-glc-5-ara, Pn-3-glc, Pg-3,5-glc, Cy-35-glc and Cy-3-glc were detected among nine wild herbaceous peony (Jia et al. 2008). Dp-3-gal, Dp-3-glc, Cy-3-gal, Cy-3-glc, Pt-3-glc, Pl-3-glc, Cy, catechin-cy-3-glucoside and Pn-3-glc were identified in black soybean (Lee et al. 2009). In all the above reviewed studies, commonly cyanidin and delphidin are obtained as major groups of anthocyanin, which was the case in the present study also. The most abundant anthocyanin in coloured cereal grains are reported to be Cy 3-glu, Pg-3-gal, Pn-3-glu (Naczk and Shahidi 2006; Prior and Wu 2006). Cy-3-glc is the most predominant anthocyanin on purple corncob, purple wheat and black soybean (Jing et al 2007; Hosseinian et al. 2008; Lee et al 2009). In mulberry and *Liriope platyphylla* fruit Pt-3-rut is the most predominant anthocyanin followed by Cy-3-glc (Wang 2014). However, in *Chakhao Poireiton* and *Chakhao Amubi*, Dl-3-gal is the most predominant anthocyanin.

4.2 Total phenolics, total anthocyanin content and the DPPH assay

The pH differential method is a rapid and easy procedure for the quantification of monomeric anthocyanin (Giusti and Wrolstad 2001). Anthocyanin pigments undergo reversible structural transformations with a change in pH manifested by strikingly different absorbance spectra. The colored oxonium form predominates at pH 1.0 and the hemiketal (colourless) form at pH 4.5. The pH differential method is based on this reaction and permits accurate and rapid measurements for the total amount of anthocyanin, even in the presence of polymerized degraded pigments & other interfering compounds (Giusti and Wrolstad 2001). In the study, two black scented rice cultivars (*Chakhao Poireiton* and *Chakhao Amubi*) were taken. The levels of monomeric anthocyanins was found to be 740 mg/kg in *Chakhao Poireiton* and 692 mg/kg in *Chakhao Amubi*. The results were consistent with the results reported for purple wheat (526.0 and 500.6 mg/kg) (Hosseinian et al. 2008) and 1214.85 mg /kg of black rice (Park et al. 2008). And the phenolic contents were measured using a modified Folin–Ciocalteu method giving the value 577 and 500 mg/100 g of the powdered sample as gallic acid equivalents in *Chakhao Poireiton* and *Chakhao Amubi*, respectively. The presence of phenolics show antioxidant activity as phenolic compounds is a class of antioxidant agents which act as free radical terminators and their bioactivities may be related to their abilities to chelate metals, inhibit lipoxygenase and scavenge free radicals (Roya and Fatemeh 2013).

The antioxidant activity of the black scented rice has been determined using the DPPH assay and showed very strong antioxidant activity. The DPPH highest free radical scavenging activity of *Chakhao Poireiton* and *Chakhao Amubi* are 70.28 and 60.84 %, respectively. Although, scavenging activity is a little lower than the standard ascorbic acid, the anthocyanin extract showed strong antioxidant activity. The result has been consistent with work on Korean black rice (*Heugjunjubyeo*), the DPPH radical scavenging capacity of anthocyanin extract exhibited 40.39 and 55.20 % scavenging activity at 50 and 100 μg/mL, respectively (Park et al. 2008). The study of Saenkod et al. 2013, also showed the Chinese black rice (Brown Himi variety) has strong antioxidant activity which 70.82 % DPPH scavenging activity and the total phenolics was 634.83 mg/Kg. The red rice from Minahasa, North Sulawasi, Indonesia reported 88.29 % DPPH scavenging activity and 58.55 mg/g anthocyanin content (Moko et al. 2014). Antioxidant capacity is becoming a parameter to characterize food or medicinal plants and their bioactive components. Higher percentage of DPPH scavenging shows higher antioxidant capacity (Sultana et al. 2009). In the human body, dietary antioxidants protect against reactive oxygen species (Saenkod et al. 2013). Thus, if the anti-oxidants intake is increased, there may have a number of health effects, such as reducing the incidence of cancer and cardiovascular diseases (Diplock et al. 1998). Due to the antioxidant activity of anthocyanins, they have been recognized as health promoting food ingredients, recently (Nam et al. 2006; Philpott et al. 2006), and anticancer (Hyun and Chung 2004), hypoglycemic

(Tsuda et al. 2003), and anti-inflammatory effects (Tsuda et al. 2002). It has been also reported that anthocyanins may reduce the risks of cardiovascular diseases and cancer with antiinflammatory, antioxidant and chemoprotective properties (Park et al. 2008). In mice, the supplementation of black rice pigment in the diet reduced oxidative stress (Xia et al. 2003) and its pigment fraction may have antiatherogenic activity (Xia et al. 2006). The black rice is a good source of fiber, minerals, and several important amino acids (Zhang et al. 2005), and there is an increased interest in the alternative sources of anthocyanins due to a rising demand for economical sources of natural and stable pigments (Hu et al. 2003). Many new bio-active compounds have been detected in many source of food with possible antioxidant activity and the increased interest in the relationship between antioxidants and disease risk mechanisms, there is an urgent need to establish the antioxidant capacity in different foods, especially the rice crop which constitutes the main food for populations in different countries (Saenkod et al. 2013).

Despite the use of plant extracts in medicine and food, recently it has been used in plant protection. Plant secondary metabolites include phenol, phenolic acids, quinines, flavones, flavonoids, flavonols, tannins and coumarins, many previous reports showed that these compounds have plant defensive mechanisms against pathogenic microorganisms. Gurjar et al. 2012, reported that simple phenols act on the membrane disruption and deprive the substrate, phenolic acids and flavonoids bind to adhesions complex with cell wall and inactivate enzymes, thus, protect from the bacteria, fungus attacks. In addition to the beneficial effects of anthocyanins, anthocyanins have also been observed to function in a diverse array of plant/animal interactions which include the attraction of pollinators and frugivores and the repellence of herbivores and parasites (Lev-Yadun and Gould 2009). They suggested that the optical properties of anthocyanins may serve as visual signals to potential herbivores, indicating a strong metabolic investment in toxic or unpalatable chemicals and they have also been implicated in the camouflage of plant parts against their backgrounds, in the undermining of insect crypsis and in the mimicry of defensive structures. Singh and Sharma 1998, reported the *Chkahao* in common are resistant to many rice diseases (sheath rot, foot rot, stem rot, narrow brown spot, false smut, bacterial leaf blight and bacterial leaf streak) and rice insect pests (stem borer, case worm, thrips, rice skipper, green horned caterpillar, green semi looper and rice bug). The resistance may be due to the present of high phenolic and anthocyanin contents. Fasahat et al. 2012, also reported that the colored rice *Oryza rufipogon* of Malaysia which content anthocyanin pigment is highly resistant to bacterial leaf blight and brown plant hopper. Three glycoflavones schaftoside, isoschaftoside and neo- schaftoside have been identified in the phloem sap of rice plants, where they act as sucking deterrents to the pest insect, the brown plant hopper *Niloparvata lugens* (Harborne and Williams 2000). In the resistant cultivars of rice, high levels of these glycoflavones are present and when tested at these concentrations on plant hoppers, they exhibited an ingestion inhibiting activity (Grayer and Harborne 1994). The stem nematode, *Ditylenchus angustus* is another pest of the rice plant, a flavonoid and a related phenylpropanoid in the leaves have been recognised as providing resistance to nematode attack. The concentrations of flavanone sakuranetin increased, after 5 days of inoculation with the nematode in the resistant cultivar whereas no changes in secondary chemistry occurred in a susceptible cultivar of rice (Plowright et al. 1996). SimilarIy, Dillon et al. 1997, observed that the same flavanone, sakuranetin, is formed in rice in response to UV-irradiation or to fungal infection and hence is also involved, in part, in protecting rice plants from plant diseases. The accumulation of flavonoids, carotenoids and betalains caused the coloration of plant organs, such as leaves, flowers and fruits (Joseph et al. 1998). In the development of the plant, in protection against UV radiation, attraction of insects for pollination and plant defense responses, both the flavonoids and flavonoid derivatives play important roles (Harborne and Williams 2000; Winkel-Shirley 1996). Flavonoids make some contribution to disease resistance, either as constitutive antifungal agents or as phytoalexins and the simple phenolic constituents or the polymeric favolans or proanthocyanidins, provide defense against herbivory (Harborne and Williams 2000). Flavonoids are also important in UV-B protection which has been shown in the experiment on *Arabidopsis thaliana*, where mutants which lack the epidermal flavonoids of the wild type plant become very sensitive to artificial UV-B radiation (Ormrod et al. 1995). *Arabidopsis* mutants which are blocked in the biosynthesis of related phenylpropanoids based on sinapic acid, are less affected by UV-B radiation (Chapple et al. 1992). A mutant of barley, Hordeum vulgare has been produced which contains only 7 % of the flavonoids of the wild type when treated with UV-B there is a decreased in the quantum yield of photosynthesis in the plant from that of the wild type plant. The existence of phenolic compounds in free form are toxic and less common but detoxified, when bound at least in part, hence, phenolic compounds play a role of protection against insects and other animals to the plants (Harborne and Williams 2000). Certain phenolic compounds and flavonoid such as the anthocyanin has been demonstrated to act as antifeedants, toxins, warning signals or precursors to physical defense systems (Harborne 1997). Recently, plant extracts which are rich in phenolic compounds are being used as phytochemicals to protect against several plant diseases like bacterial blight, stem rot, brown rot, root rot and brown spot (Enyiukwu et al. 2013). Thus, there is no wrong to suggest that the high phenolic and the antioxidant of the black scented rice act as a plant defensive mechanism which protect themselves from certain disease and pests which destroy the crop production.

In conclusion, we characterized the anthocyanins profile of black scented rice (*Chakhao Poireiton* and *Chakhao Amubi*). The present study documented the presence of four main anthocyanins including delphinidin 3-galactoside, delphinidin 3-arabinoside, cyanidin 3-galactoside and cyanidin 3-glucoside in the extract of black scented rice grains. Among the four anthocyanins, delphinidin 3-galactoside reported the most abundant. Both the cultivars (*Oryza sativa* cv. *Chakhao Poireiton* and *Chakhao Amubi*) in the study showed high anthocyanins, phenolic content and strong antioxidant activity. Therefore, the black scented rice extracts can be a potential source of antioxidative phytochemicals and useful ingredient for nutraceutical or functional food products. The supplementation of the black scented rice (*Chakhao*) in the diet will have a great impact on human health. In addition, it can be concluded that the black scented rice (*Chakhao*) has a defensive mechanism which can protect it against some of the diseases and pests as a result of the high anthocyanins and phenolic content. The anthocyanin pigment benefit the human health and are involved in the plant's defensive mechanisms. The current research on the importance of these cultivars should encourage the agricultural scientists to include them in the crop improvement programmes, research could increase productivity without losing grain quality characteristics and result in pharmaceutical applications as well as increasing our knowledge of plant defense mechanisms.

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