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Antioxidant potential of brans of twenty-nine red and white rice (*Oryza sativa* L.) varieties of Sri Lanka

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ABSTRACT

Objective: To evaluate antioxidant properties of brans of twenty-nine red and white rice varieties of Sri Lanka.

Methods: Brans of 21 new improved (NI), 2 old improved (OI) and 6 traditional red and white rice varieties of Sri Lanka were studied for range of antioxidant properties. The studied antioxidant properties included total polyphenolic content (TPC), ferric reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity and 1,1-diphenyl-2-picrylhydrazine (DPPH) radical scavenging activity *in vitro*. Bran of black rice variety from Korea was also studied for the same antioxidant properties for comparison.

Results: Results exhibited significantly high ABTS and DPPH radical scavenging activities and 10, 7 and 2.5 fold greater TPC, FRAP and ORAC activities in brans of red rices (BRRs) compared to brans of white rices irrespective of NI, OI and traditional rice types. Among BRRs traditional varieties had greater ABTS and DPPH radical scavenging activities and 1.7, 1.3 and 1.2 fold respectively greater TPC, FRAP and ORAC in contrast to NI red rices. Traditional red rice varieties, Kalu Heeneti (TPC and ORAC), Pachchaperumal (TPC and DPPH) and Kurulu Thuda (DPPH) and OI red rice variety H4 (FRAP) exhibited the highest activities for the antioxidant properties studied. Further, these varieties had significantly high activities compared to black rice.

Conclusions: In conclusion, BRRs especially traditional red rices had greater antioxidant properties and consumption may be useful in managing various chronic diseases.

1. Introduction

Free radicals generated in living organisms are neutralized through various pathways and maintain the radical concentration below harmful level[1,2]. However, when the production of free radicals exceeds its neutralization process, it causes a condition known as the oxidative stress[1,2]. It is very well documented that oxidative stress causes damage to cellular macromolecules leading to variety of chronic diseases[1-3]. Antioxidants are compounds which provide protection to cells and tissues from free radical damage and therefore important in the prevention and management of such diseases[1,2]. Research findings have clearly shown that naturally occurring antioxidants in plant foods are safe, cheap and better alternatives to many synthetic antioxidants[3,4].

Rice is one of the major cereal crops and grown over hundred countries and on every continent[5]. White rice is the most popular and widely consumed rice type worldwide. However, there are pigmented rices which contain brown, red, purple and black pigments in the outer layers of the rice grain. Brans of pigmented rices are potent sources of naturally occurring antioxidants[6-11]. In Sri Lanka, rice is the dietary staple and one of the most important food crops in the country[12,13]. Up to 1950s, the rice varieties used for cultivation in Sri Lanka were exclusively traditional types[12]. There were > 2 400 traditional rice varieties cultivated under diverse agroecological conditions[14]. As a result of rice varietal development programme in the country, a series of old improved (OI) rice varieties (H varieties) were emerged in the decade of 1960s. Further, rice breeding efforts in the country were able to develop high yielding new improved (NI) rice varieties and these

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varieties were dominated after 1980s. Currently 99% extent of the paddy cultivation in the country comprises NI rice varieties and more than 50 such NI rice varieties are cultivated island-wide[12].

To date the studies on antioxidant properties of widely cultivating and consuming NI rice varieties and OI rice varieties are extremely limited. Further, there are limited studies on antioxidant properties of Sri Lankan traditional rice[13,15] although these varieties are traditionally claimed to have variety of health benefits[14,16]. Furthermore, comparative studies on antioxidant properties of traditional, OI and NI rice varieties are important to identify the best rice varieties for consumption and also for selection of parental materials for rice breeding programme in the country. This study investigated potential of brans of large set of NI and selected OI and traditional rice varieties of Sri Lanka for range of antioxidant properties.

2. Materials and methods

2.1. Grain samples

Twenty-one NI, 2 OI and 6 traditional rice varieties of Sri Lanka were obtained from Rice Research and Development Institute, Bathalagoda, Sri Lanka. A whole grain black rice variety collected from the local market of Korea was also used for the comparison.

2.2. Chemicals and reagents

6-Hydroxy-2,5,7,8-tetramethylchroman-2carboxylic acid (Trolox), gallic acid, 1,1-diphenyl-2-picrylhydrazine (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) diammonium salt, 2,4,6-tripyridyl-s-triazine (TPTZ), potassium persulfate, ferric chloride, fluorescein, 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) and Folin-Ciocalteu phenol reagent were purchased from Sigma-Aldrich, USA. All the other chemicals used for the preparation of buffers and solvents were of analytical grade.

2.3. Sample preparation

Rice seeds were dehulled using a laboratory dehuller (THU 35B, Satake, Hiroshima, Japan). Dehulled grains were polished in a laboratory polisher (TM-05C, Satake, Hiroshima, Japan) and rice bran was passed through a 60 mesh sieve to obtain a uniform fraction of rice bran.

2.4. Extraction of rice bran

One gram of rice bran was extracted with 10 times the sample weight of 70% ethanol water (v/v) overnight at room temperature [(28 ± 2) ℃]. Then, rice bran extracts were centrifuged (3 500 r/min) for 10 min and filtered through 0.45 μm nylon filters and evaporated to dryness under vacuum in a rotary evaporator and freeze dried (Christ-Alpha 1–4 Freeze dryer, Biotech International, Germany). The freeze dried rice bran extracts were used in evaluation of antioxidant properties.

2.5. Total polyphenolic content (TPC)

The TPC of rice bran extracts was determined according to the method of Singleton *et al.*[17] using 96 well microplates (n = 3). Twenty microliters of rice bran extracts (1 mg/mL) was added to 110 μL of 10 times diluted freshly prepared Folin-Ciocalteu reagent. Then, 70 μL of sodium carbonate solution was added, incubated at 25 ℃ for 30 min and the absorbance was recorded at 765 nm using a 96 well microplate reader (SpectraMax Plus 384, Molecular Devices, USA). Gallic acid was used as the standard antioxidant. TPC was expressed as mg gallic acid equivalents (GAE)/100 g dry weight of rice bran.

2.6. Ferric reducing antioxidant power (FRAP)

FRAP of rice bran extracts was carried out according to the method of Benzie and Szeto[18] in 96 well microplates (n = 3). The FRAP reagent was produced by mixing 300 mmol/L acetate buffer (pH 3.6), 10 mmol/L TPTZ solution and 20 mmol/L $FeCl_3.6H_2O$ in a ratio of 10:1:1. Then, the solution was heated at 37 ℃ for 10 min. The TPTZ solution was prepared by making a solution of 10 mmol/L TPTZ in 40 mmol/L HCl. Reaction volume of 200 μL containing 150 μL working FRAP reagent, 30 μL acetate buffer and 10 μL of rice bran extracts (white rice: 1 mg/mL, red and black rice: 0.5 mg/mL) were incubated at (25 ± 2) ℃ for 8 min. The absorbance was recorded at 600 nm using a 96 well microplate reader. Trolox was used as the standard antioxidant. Results were expressed as mg Trolox equivalents (TE)/g bran in dry weight basis.

2.7. Oxygen radical absorbance capacity (ORAC)

The ORAC of rice bran extracts was carried out according to the method described by Ou *et al.*[19] with some modifications in 96 well microplates. Reaction volume of 200 μL, containing 100 μL of fluorescein (4.8 μmol/L) and 50 μL of rice bran extracts (1 mg/mL; n = 3 each) was pre-incubated at 37 ℃ for 10 min. Then, the reaction was initiated by addition of 50 μL of AAPH (40 mg/mL). The fluorescein and AAPH solutions were prepared in 75 mmol/L phosphate buffer (pH 7.4). The decay of fluorescein was measured for 35 min at excitation and emission wave lengths of 494 nm and 535 nm respectively in 1 min interval using a fluorescent microplate reader (SpectraMax-Gemini EM, Molecular Devices Inc, USA). Trolox was used as the standard antioxidant. Results were expressed as mg TE/g dry weight of rice bran.

2.8. ABTS radical scavenging activity

The ABTS radical scavenging activity of rice bran extracts was carried out according to the method of Re *et al.*[20] with some modifications in 96 well microplates. Reaction volume of 200 μL, containing 40 μL of seven times diluted ABTS stock solution (7.8 mmol/L of ABTS in potassium persulfate), 5 μL of rice bran extracts (1 mg/mL; screening: n = 3 each) and 155 μL of 0.1 mol/L phosphate buffer was incubated at (25 ± 2) ℃ for 10 min. The absorbance readings were then recorded at 734 nm using a 96 well microplate reader. For dose response studies, different concentrations (1.56, 3.12, 6.25, 12.5 and 25 μg/mL; n = 3 each) of bran extracts of selected rice were used. Trolox was used as the standard antioxidant. Results were expressed as % inhibition and IC_{50} values.

2.9. DPPH radical scavenging activity

The DPPH radical scavenging activity of rice bran extracts

was carried out according to the method of Blois[21] with some modifications in 96 well microplates. Reaction volume of 200 µL, containing 40 µL (20 mg/100 mL) of DPPH radical, 5 µL of rice bran extracts (1 mg/mL; screening: n = 3 each) and 155 µL of methanol was incubated at (25 ± 2) ˚C for 15 min. Then, absorbance was read at 517 nm using a 96 well microplate reader. Selected rice varieties were studied for dose response using series of different rice bran concentrations (3.12, 6.25, 12.5, 25 and 50 µg/mL; n = 3 each). Trolox was used as the standard antioxidant. Results were expressed as % inhibition and IC_{50} values.

2.10. Statistical analysis

Results presented as mean ± SE. SAS version 6.12 was used in the statistical analysis of data. One-way ANOVA was used in data analysis. Differences among treatment means were performed using Duncan's multiple range test. The Pearson's correlation coefficient was used for the correlation analysis. $P < 0.05$ was regarded as significant.

3. Results

3.1. TPC

TPC of the investigated rice varieties and range of TPC among different rice types are given in Tables 1 and 2 respectively. Significant differences ($P < 0.05$) were observed for TPC among the varieties and among different rice types. Mean TPC of selected rice varieties ranged from (21.91 ± 2.68) to (2 808.14 ± 26.77) mg GAE/100 g bran. Traditional red rice variety Kalu Heeneti exhibited the highest TPC, while a NI white rice variety Bg 250 showed the lowest. Mean TPC of traditional, OI and NI rice varieties ranged from (157.55 ± 1.54) to (2 808.14 ± 26.77), (84.25 ± 3.69) to (1 479.51 ± 17.19) and (21.91 ± 2.68) to (1 810.93 ± 15.84) mg GAE/100 g bran respectively. Results clearly demonstrated that irrespective of rice types brans of red rices had significantly high ($P < 0.05$) TPC than brans of white rices. Further, brans of traditional red rices exhibited significantly high ($P < 0.05$) TPC than brans of NI red rices. Furthermore, brans of Kalu Heeneti, Pachchaperumal and Beheth Heeneti exhibited significantly high TPC ($P < 0.05$) than TPC of brans of black rice [(2 034.03 ± 7.79) mg GAE/100 g bran].

3.2. FRAP

FRAP of rice varieties and range of FRAP among different rice types are given in Tables 1 and 2 respectively. Results revealed significant differences ($P < 0.05$) among the varieties and among different rice types for FRAP. Mean FRAP of rice varieties tested ranged from (1.71 ± 1.37) to (58.01 ± 0.64) mg TE/g bran. OI red rice variety H4 exhibited the highest FRAP, while NI white rice variety Bg 305 had the lowest. Mean FRAP of different rice types: traditional, OI and NI ranged from (2.60 ± 0.05) to (46.23 ± 1.00), (6.04 ± 0.04) to (58.01 ± 0.64) and (1.71 ± 1.37) to (34.16 ± 0.78) mg TE/g bran respectively. Results clearly showed that irrespective of different rice types brans of red rices had significantly high ($P < 0.05$) FRAP than brans of white rices. Further, FRAP of brans of traditional red rices was significantly high ($P < 0.05$) compared to NI red rices. The order of potency of red rices for FRAP was H4 > Kalu

Table 1
Antioxidant properties of brans of twenty-nine rice varieties of Sri Lanka.

No	Variety	Pericarp color	Rice type	TPC	FRAP	ORAC	ABTS	DPPH
1	Kalu Heeneti	Red	Traditional	2 808.14 ± 26.77^{a}	46.23 ± 1.00^{b}	26.63 ± 0.23^{a}	96.87 ± 0.53^{a}	59.28 ± 2.57^{b}
2	Pachchaperumal	Red	Traditional	2 489.63 ± 47.08^{b}	31.52 ± 0.49^{f}	22.02 ± 0.73^{c}	97.29 ± 0.11^{a}	68.58 ± 3.88^{a}
3	Beheth Heeneti	Red	Traditional	2 070.96 ± 20.73^{c}	40.86 ± 0.63^{c}	22.51 ± 0.58^{bc}	98.28 ± 0.40^{a}	68.63 ± 5.50^{a}
4	Kurulu Thuda	Red	Traditional	1 855.38 ± 14.66^{d}	40.59 ± 1.10^{c}	22.41 ± 0.59^{bc}	98.10 ± 0.08^{a}	59.33 ± 0.39^{b}
5	At 353	Red	NI	1 810.93 ± 15.84^{d}	34.16 ± 0.78^{e}	23.54 ± 0.77^{b}	78.35 ± 1.10^{b}	41.09 ± 2.75^{c}
6	Dosthara Heeneti	Red	Traditional	1 670.71 ± 71.48^{c}	36.55 ± 0.96^{d}	21.40 ± 0.78^{c}	98.22 ± 0.42^{a}	57.22 ± 3.62^{b}
7	H4	Red	OI	1 479.51 ± 17.19^{f}	58.01 ± 0.64^{a}	17.07 ± 0.54^{d}	81.91 ± 5.60^{b}	61.15 ± 5.34^{b}
8	At 362	Red	NI	1 156.02 ± 10.80^{g}	28.05 ± 0.02^{g}	18.17 ± 1.78^{d}	64.85 ± 7.68^{c}	45.37 ± 2.67^{c}
9	Bg 406	Red	NI	840.36 ± 32.33^{h}	25.70 ± 0.79^{h}	15.41 ± 0.28^{e}	50.72 ± 1.97^{d}	33.16 ± 1.02^{d}
10	Bg 358	white	NI	328.83 ± 4.77^{i}	6.22 ± 0.08^{kl}	9.59 ± 0.18^{i}	23.19 ± 1.69^{hijk}	4.55 ± 0.53^{i}
11	Bg 357	white	NI	325.11 ± 17.17^{ij}	6.21 ± 0.08^{kl}	7.35 ± 1.15^{jk}	17.08 ± 2.30^{kl}	15.73 ± 5.34^{efg}
12	Bg 352	white	NI	278.43 ± 11.00^{jk}	6.32 ± 0.16^{jkl}	13.53 ± 0.21^{f}	26.39 ± 1.04^{fghi}	21.84 ± 2.18^{e}
13	Bg 360	white	NI	266.16 ± 4.83^{kl}	4.14 ± 0.06^{m}	5.98 ± 0.56^{k}	31.00 ± 4.01^{efg}	6.63 ± 1.10^{hi}
14	Bg 379-2	white	NI	262.77 ± 8.69^{lk}	5.59 ± 0.07^{kl}	11.66 ± 0.54^{g}	22.55 ± 2.19^{hijk}	7.32 ± 0.68^{hi}
15	Bg 450	white	NI	259.42 ± 13.12^{lkm}	5.96 ± 0.18^{kl}	11.33 ± 0.09^{gh}	31.36 ± 1.06^{ef}	4.61 ± 1.28^{i}
16	Bg 454	white	NI	216.63 ± 3.74^{lm}	5.41 ± 0.08^{l}	9.68 ± 0.55^{i}	27.04 ± 0.92^{fghi}	14.24 ± 0.97^{efgh}
17	Bg 3-5	white	NI	214.64 ± 2.89^{lm}	7.53 ± 0.10^{j}	7.68 ± 0.44^{j}	36.63 ± 1.64^{e}	9.12 ± 0.72^{ghi}
18	At 405	white	NI	209.76 ± 2.70^{m}	4.15 ± 0.07^{m}	7.36 ± 0.75^{jk}	23.51 ± 1.09^{hijk}	5.25 ± 0.72^{i}
19	Rathdhal	white	Traditional	157.55 ± 1.54^{n}	2.60 ± 0.05^{no}	3.81 ± 0.09^{l}	28.34 ± 4.86^{fgh}	5.02 ± 0.55^{i}
20	Bg 300	white	NI	155.81 ± 6.23^{n}	11.07 ± 0.18^{i}	11.77 ± 0.52^{g}	30.46 ± 1.39^{fg}	16.41 ± 5.40^{efg}
21	Bg 407	white	NI	134.53 ± 2.79^{on}	5.79 ± 0.04^{kl}	9.26 ± 0.76^{i}	26.43 ± 1.78^{fghi}	7.23 ± 1.24^{hi}
22	At 307	white	NI	114.46 ± 5.01^{on}	10.84 ± 0.22^{i}	13.64 ± 0.77^{f}	24.62 ± 0.98^{ghij}	12.16 ± 2.89^{fghi}
23	At 306	white	NI	111.72 ± 5.57^{onp}	2.88 ± 0.06^{mno}	6.19 ± 0.18^{jk}	18.26 ± 1.40^{jkl}	18.67 ± 0.64^{ef}
24	Bg 305	white	NI	105.63 ± 2.09^{onp}	1.71 ± 1.37^{o}	7.41 ± 0.28^{jk}	21.33 ± 2.30^{ijk}	14.55 ± 2.44^{efgh}
25	Bg 369	white	NI	94.28 ± 8.25^{oqp}	6.81 ± 0.12^{jk}	6.83 ± 0.47^{jk}	26.10 ± 2.76^{fghi}	18.25 ± 3.63^{ef}
26	H7	white	OI	84.25 ± 3.69^{oqp}	6.04 ± 0.04^{kl}	10.20 ± 1.36^{hi}	15.01 ± 1.70^{l}	8.71 ± 0.21^{ghi}
27	Bg 400-1	white	NI	60.67 ± 2.25^{rqp}	3.34 ± 0.03^{mn}	4.46 ± 0.27^{l}	21.75 ± 1.11^{hijk}	12.91 ± 1.53^{fghi}
28	Bg 94/1	white	NI	50.48 ± 3.57^{rq}	3.85 ± 0.07^{mn}	4.45 ± 0.33^{l}	24.51 ± 3.00^{ghij}	13.95 ± 5.82^{efgh}
29	Bg 250	white	NI	21.91 ± 2.68^{r}	2.76 ± 0.05^{no}	6.82 ± 0.28^{jk}	17.18 ± 1.75^{kl}	12.50 ± 0.64^{fghi}

Data represented as mean ± SE (n = 3 each). Mean values in a column superscripted by different letters are significantly different at $P < 0.05$. TPC: mg GAE/100 g bran; FRAP: mg TE/g bran; ORAC: mg TE/g bran; ABTS radical scavenging activity, % inhibition at 25 µg/mL; DPPH radical scavenging activity, % inhibition at 25 µg/mL.

Table 2
Variation in antioxidant properties of brans of different rice types of Sri Lanka.

Rice type		TPC	FRAP	ORAC	ABTS	DPPH
Traditional	All*	157.55 ± 1.54 – 2808.14 ± 26.77	2.60 ± 0.05 – 46.23 ± 1.00	3.81 ± 0.09 – 26.63 ± 0.23	28.34 ± 4.86 – 98.28 ± 0.40	5.02 ± 0.55 – 68.63 ± 5.50
	Red	1670.71 ± 71.48 – 2808.14 ± 26.77	31.52 ± 0.49 – 46.23 ± 1.00	21.40 ± 0.78 – 26.63 ± 0.23	96.87 ± 0.53 – 98.28 ± 0.40	57.22 ± 3.62 – 68.63 ± 5.50
	White	157.55 ± 1.54	2.60 ± 0.05	3.81 ± 0.09	28.34 ± 4.86	5.02 ± 0.55
OI	All*	84.25 ± 3.69 – 1479.51 ± 17.19	6.04 ± 0.04 – 58.01 ± 0.64	10.20 ± 1.36 – 17.07 ± 0.54	15.01 ± 1.70 – 81.91 ± 0.20	8.71 ± 0.21 – 61.15 ± 5.34
	Red	1479.51 ± 17.19	58.01 ± 0.64	17.07 ± 0.54	81.91 ± 0.20	61.15 ± 5.34
	white	84.25 ± 3.69	6.04 ± 0.04	10.20 ± 1.36	15.01 ± 1.70	8.71 ± 0.21
NI	All*	21.91 ± 2.68 – 1810.93 ± 15.84	1.71 ± 1.37 – 34.16 ± 0.78	4.45 ± 0.33 – 23.54 ± 0.77	17.08 ± 2.30 – 78.35 ± 1.10	4.55 ± 0.53 – 45.37 ± 2.67
	Red	840.36 ± 32.33 – 1810.93 ± 15.84	25.70 ± 0.79 – 34.16 ± 0.78	15.41 ± 0.28 – 23.54 ± 0.77	50.72 ± 1.97 – 78.35 ± 1.10	33.16 ± 1.02 – 45.37 ± 2.67
	White	21.91 ± 2.68 – 328.83 ± 4.77	1.71 ± 1.37 – 11.07 ± 0.18	4.45 ± 0.33 – 13.64 ± 0.77	17.08 ± 2.30 – 36.63 ± 1.64	4.55 ± 0.53 – 21.84 ± 2.18
Red rice		840.36 ± 32.33 – 2808.14 ± 26.77	25.70 ± 0.79 – 58.01 ± 0.64	15.41 ± 0.28 – 26.63 ± 0.23	50.72 ± 1.97 – 98.28 ± 0.40	33.16 ± 1.02 – 68.63 ± 5.50
White rice		21.91 ± 2.68 – 328.83 ± 4.77	1.71 ± 1.37 – 11.07 ± 0.18	3.81 ± 0.09 – 13.64 ± 0.77	15.01 ± 1.70 – 36.63 ± 1.64	4.55 ± 0.53 – 21.84 ± 2.18

Data represented as mean ± SE (n = 3 each). TPC: mg GAE/100 g bran; FRAP: mg TE/g bran; ORAC: mg TE/g bran; ABTS radical scavenging activity, % inhibition at 25 μg/mL; DPPH radical scavenging activity, % inhibition at 25 μg/mL. *: Both red and white varieties.

Heeneti > Beheth Heeneti = Kurulu Thuda > Dosthara Heeneti > At 353 > Pachchaperumal > At 362 > Bg 406. Interestingly, FRAP of brans of H4 was significantly high ($P < 0.05$) compared to the FRAP of brans of black rice [(45.36 ± 0.95) mg TE/g bran] while Kalu Heeneti showed comparable activity.

3.3. ORAC

ORAC of brans of selected rice varieties and range of ORAC among different rice types are given in Tables 1 and 2 respectively. Results revealed significant differences ($P < 0.05$) in ORAC among the varieties and it ranged from (3.81 ± 0.09) to (26.63 ± 0.23) mg TE/g of bran. Bran extract of traditional red rice variety Kalu Heeneti exhibited the highest ORAC, while a traditional white rice variety Rathdal had the lowest. The mean ORAC of traditional, OI and NI rice types ranged from (3.81 ± 0.09) to (26.63 ± 0.23), (10.20 ± 1.36) to (17.07 ± 0.54) and (4.45 ± 0.33) to (23.54 ± 0.77) mg TE/g of bran respectively. Irrespective of different rice types brans of red rices exhibited significantly high ($P < 0.05$) ORAC compared to brans of white rices. Further, brans of traditional red rices showed significantly high ($P < 0.05$) ORAC compared to brans of NI red rices. Interestingly, brans of traditional red rice variety Kalu Heeneti exhibited significantly high ($P < 0.05$) ORAC than ORAC of brans of black rice [(24.51 ± 0.65) mg TE/g bran].

3.4. ABTS radical scavenging activity

Percent ABTS radical scavenging activity of brans of 29 rice varieties (25 μg/mL) and dose response relationship of selected red rice varieties are given in Tables 1 and 3 respectively. Percent inhibitory activity ranged from 15.01% ± 1.70% to 98.28% ± 0.40% among all the varieties studied. Inhibitory activity of traditional, OI and NI rice types ranged from 28.34% ± 4.86% to 98.28% ± 0.40%, 15.01% ± 1.70% to 81.91% ± 0.20% and 17.08% ± 2.30% to 78.35% ± 1.10% respectively. Irrespective of different rice types brans of red rices exhibited significantly high ($P < 0.05$) ABTS radical scavenging activity compared to brans of white rices. Further, brans of red rices demonstrated significant differences ($P < 0.05$) in dose response studies. Brans of traditional red rice variety Dosthara Heeneti had the highest ABTS radical scavenging activity while NI Bg 406 had the lowest. Interestingly, brans of Dosthara Heeneti, Pachchaperumal and Kalu Heeneti demonstrated comparable inhibitory activity to the brans of black rice [IC_{50}: (11.54 ± 0.53) μg/mL].

3.5. DPPH radical scavenging activity

DPPH radical scavenging activity of brans of 29 rice varieties (25 μg/mL) and dose response relationship of selected red rice varieties are given in Tables 1 and 4 respectively. Inhibitory activity varied from 4.55% ± 0.53% to 68.63% ± 5.50% among all the rice varieties studied. Traditional, OI and NI rice types had inhibitory activities in the range of 5.02% ± 0.55% to 68.63% ± 5.50%, 8.71% ± 0.21% to 61.15% ± 5.34% and 4.55% ± 0.53% to 45.37% ± 2.67% respectively. Irrespective of different rice types brans of red rices exhibited significantly high ($P < 0.05$) inhibitory activity compared to brans of white rices. Further, among brans of red rices significant differences ($P < 0.05$) were observed. Traditional (Beheth Heeneti, Kurulu Thuda, Pachchaperumal, Kalu Heeneti, Dosthara Heeneti) and OI (H4) red rices exhibited significantly high ($P < 0.05$)

Table 3
Dose response relationship of brans of selected red rice varieties of Sri Lanka for ABTS radical scavenging activity.

Rice type	Rice variety	Concentration (μg/mL)					IC_{50} (μg/mL)
		1.56	3.12	6.25	12.5	25	
Traditional	Dosthara Heeneti	13.13 ± 1.43	19.87 ± 1.05	32.04 ± 0.63	55.93 ± 0.80	94.22 ± 0.22	11.71 ± 0.16^{f}
Traditional	Pachchaperumal	-1.75 ± 1.87	-0.10 ± 3.86	27.32 ± 5.32	55.35 ± 3.85	94.99 ± 3.44	12.49 ± 1.16^{ef}
Traditional	Kalu Heeneti	0.13 ± 1.78	0.78 ± 0.54	31.54 ± 2.42	49.77 ± 3.04	86.86 ± 1.38	13.86 ± 0.35^{e}
Traditional	Kurulu Thuda	2.05 ± 0.53	11.41 ± 5.51	15.38 ± 1.75	44.91 ± 0.57	85.98 ± 2.04	14.28 ± 0.37^{de}
OI	H4	3.02 ± 0.72	7.70 ± 0.83	20.26 ± 0.43	43.74 ± 2.69	81.62 ± 1.30	14.43 ± 0.02^{de}
Traditional	Beheth Heeneti	-8.65 ± 1.47	-2.79 ± 2.80	10.53 ± 1.94	35.24 ± 5.16	86.75 ± 3.26	16.30 ± 0.27^{cd}
NI	At 353	-3.88 ± 7.44	3.55 ± 2.47	22.11 ± 7.41	29.63 ± 10.73	77.95 ± 0.92	17.81 ± 0.08^{c}
NI	At 362	5.91 ± 0.84	9.87 ± 1.49	15.72 ± 0.68	30.96 ± 0.98	62.40 ± 2.09	22.03 ± 1.43^{b}
NI	Bg 406	3.39 ± 0.35	10.18 ± 1.55	17.53 ± 0.82	27.66 ± 1.14	47.92 ± 3.27	29.44 ± 0.84^{a}

Data represented as mean ± SE (n = 3 each). Mean IC_{50} values in a column superscripted by different letters are significantly different at $P < 0.05$. Trolox IC_{50}: (3.45 ± 0.11) μg/mL.

Table 4

Dose response relationship of brans of selected red rice varieties of Sri Lanka for DPPH radical scavenging activity.

Rice type	Rice variety	Concentration (μg/mL)					IC_{50} (μg/mL)
		3.12	6.25	12.5	25	50	
Traditional	Beheth Heeneti	12.54 ± 2.13	26.29 ± 2.54	51.61 ± 1.46	72.47 ± 5.48	92.64 ± 0.58	12.09 ± 0.31^{e}
Traditional	Kurulu Thuda	8.91 ± 0.59	20.07 ± 3.39	45.85 ± 0.67	73.79 ± 2.20	90.39 ± 0.16	13.62 ± 0.38^{e}
Traditional	Pachchaperumal	17.53 ± 1.09	25.18 ± 1.46	37.67 ± 3.06	71.45 ± 1.01	83.46 ± 0.58	16.62 ± 0.16^{de}
Traditional	Kalu Heeneti	18.87 ± 2.66	28.36 ± 3.89	42.80 ± 3.20	65.68 ± 1.79	89.26 ± 0.21	19.97 ± 1.55^{cd}
OI	H4	9.91 ± 3.42	25.10 ± 3.99	37.43 ± 2.28	61.72 ± 1.06	84.99 ± 1.25	21.55 ± 0.77^{cd}
Traditional	Dosthara Heeneti	8.41 ± 4.14	18.47 ± 4.57	34.91 ± 0.69	59.75 ± 2.25	68.32 ± 5.09	22.44 ± 3.17^{c}
NI	Bg 406	5.48 ± 0.60	8.22 ± 1.10	17.12 ± 1.80	32.52 ± 1.89	62.26 ± 2.87	39.83 ± 2.04^{b}
NI	At 362	7.07 ± 5.68	9.24 ± 6.28	20.30 ± 6.38	37.23 ± 6.26	60.95 ± 7.00	42.31 ± 3.71^{ab}
NI	At 353	0.22 ± 0.87	7.39 ± 0.97	19.92 ± 1.40	37.99 ± 4.80	53.51 ± 3.01	45.58 ± 2.09^{a}

Data represented as mean ± SE (n = 3 each). Mean IC_{50} values in a column superscripted by different letters are significantly different at $P < 0.05$. Trolox IC_{50}: (7.67 ± 0.11) μg/mL.

inhibitory activity compared to NI red rices. Interestingly, brans of Beheth Heeneti, Kurulu Thuda and Pachchaperumal exhibited significantly high activity ($P < 0.05$) than black rice [IC_{50}: (19.43 ± 1.03) μg/mL].

4. Discussion

Phenolic compounds are widely found in food plants and are reported to mediate variety of biological activities including antioxidant activity. The quantity of phenolic compounds varies depending on the plant species and among different varieties[3-5,8]. Present study showed wide variation in TPC between brans of red and white rices and among NI, OI and traditional rice types of Sri Lanka. Mean TPC of brans of red rices were 10 fold higher than the brans of white rices. Further, mean TPC of brans of NI red rices were nearly 7 fold high compared to the brans of NI white rices. The most popular NI white rice varieties Bg 300, Bg 352 and Bg 94/1 accounting nearly 45% extent of paddy cultivation in the country[12,22] had nearly 8 fold low TPC in comparison to 2 popular NI red rices At 362 and At 353 tested in this study. Among different rice types studied, traditional red rice varieties had the greatest TPC although such rice varieties account ~1% paddy production in the country[23]. TPC of brans of Kalu Heeneti (highest TPC) was 56, 18 and 10 fold high compared to NI Bg 94/1, Bg 300 and Bg 352 white varieties respectively. Further, TPC of Kalu Heeneti was greater than black rice although black rice is reported to possess greatest amounts of phenolic antioxidants compared to the other pigmented rices[7,8,24].

FRAP assay measures the total reducing power of a sample and associated with antioxidant activity[25]. FRAP between brans of red and white rices and among different rice types also showed wide variation. Nearly 7 fold high FRAP was observed in brans of red rices compared to the brans of white rices. Further, brans of traditional red rices showed 1.3 fold high FRAP compared to the brans of NI red rices. Furthermore, brans of Kalu Heeneti had 1.5 fold greater FRAP compared to NI red rices At 353 and At 362. Moreover, 12, 7.3 and 4.2 fold high FRAP was observed in brans of Kalu Heeneti in comparison to Bg 94-1, Bg 352 and Bg 300 NI white rice varieties respectively. Interestingly, OI red rice variety H4 (highest FRAP) exhibited 1.3 fold high FRAP compared to black rice.

Free radicals are involved in the development and progression of many chronic diseases[2,3]. Thus, dietary intake of foods having radical scavenging properties is important in the prevention and dietary management of such diseases[3,4]. In this study, radical scavenging activities were evaluated using both physiological and nonphysiological radicals based antioxidant assays[3,25]. The ORAC assay measures the radical scavenging activity of peroxyl radical, which is a physiological radical in nature[25]. Results showed 2.5 fold greater ORAC in brans of red rices in contrast to brans of white rices. Further, mean ORAC of brans of traditional red rices were 1.2 fold higher than brans of NI red rices. Furthermore, brans of Kalu Heeneti (highest ORAC) had greater ORAC than black rice. Among NI red rices only At 353 showed comparable ORAC to the traditional red rices Pachchaperumal, Beheth Heeneti and Kurulu Thuda. ORAC of At 353 was 5.3, 2.0 and1.7 fold respectively high in comparison to the popular NI Bg 94-1, Bg 300 and Bg 352 white rice varieties.

Radical scavenging activity evaluated using nonphysiological $ABTS^{+}$ and $DPPH^{\bullet}$ radicals[25] also exhibited significantly high activities in brans of red rices. In dose response studies for both $ABTS^{+}$ and $DPPH^{\bullet}$ radical scavenging activities brans of traditional (Dosthara Heeneti, Pachchaperumal, Kalu Heeneti, Kurulu Thuda, and Beheth Heeneti) and OI (H4) red rices showed significantly high activity compared to the brans of NI (At 353, At 362 and Bg 406) red rices. Interestingly, Beheth Heeneti, Pachchaperumal and Kurulu Thuda had greater DPPH radical scavenging activity compared to the brans of black rice. Further, brans of Dosthara Heeneti and Pachchaperumal showed comparable ABTS radical scavenging activity to the brans of black rice. Some recent studies have also shown that red rices had greater radical scavenging activity compared to black rice and this has been explained due to the varietal difference rather than red or black pigment in the rice bran[6,9].

Pair-wise correlations between TPC and different antioxidant activity assays studied showed significant positive correlations ($P < 0.05$) indicating that phenolic compounds play a vital role in antioxidant activity of the rice bran. Our findings are in agreement with the findings of Zhang *et al.*[7] and Sompong *et al.*[9]. Further, TPC had significant positive correlation ($P < 0.05$) with the pericarp color of the rice grain indicating that phenolic compounds are prominent in pigmented rices and are responsible for the antioxidant activity of the rice bran. This is in agreement with the findings by Muntana and Prasong[6], Sompong *et al.*[9] and Gunaratne *et al.*[13].

NI white rice varieties are the most popular and widely cultivating rice varieties worldwide[26]. In contrast, popularity of NI red rice varieties are limited worldwide[26] including Sri Lanka. Findings of this study highlighted the great difference in antioxidant properties in brans of NI red rices in comparison to NI white rices. Further, findings clearly showed enhanced antioxidant properties in traditional red rice varieties in contrast to NI red rice varieties. Traditional red grain varieties had long been consumed in some Asian countries including Sri Lanka[13,26] and claimed for its superior nutritional

quality and enhanced health benefits[14,16]. Findings from the present study and our previous studies were able to prove such traditional health claims scientifically[15,16,27,28]. Further, this is the first study to demonstrate differences in antioxidant properties of large set of NI rice varieties in comparison to OI and traditional rice varieties of Sri Lanka. Traditional rice farming is environmentally friendly, economically viable, sustainable and socially acceptable[14]. Thus, a national effort is required to promote the cultivation of red grain traditional rice varieties. Further, consumption of red rices especially traditional red rices with the bran may be important in prevention and dietary management of variety of chronic diseases.

It is concluded that brans red rices exhibited greater antioxidant properties compared to brans of white rices. Further, brans of traditional red rices had greater antioxidant properties in contrast to brans of NI red rices. Furthermore, antioxidant properties of brans of some traditional red rices were greater or comparable with the antioxidant properties of black rice.

Conflict of interest statement

We declare that we have no conflict of interest.

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