Journal of Pharmaceutical Research International

32(38): 39-46, 2020; Article no.JPRI.63773
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Assessment of Antioxidant Potential of Lutein, a Retinol Equivalent Carotenoid in Medicinal Landrace of Rice 'Kavuni'

Suvarna Rani Chimmili[1*], C. R. Ananda Kumar[2], G. Subashini[2], M. Raveendran[3], S. Robin[2], G. Padmavathi[1], J. Aravind Kumar[1], B. Jyothi[1], B. Divya[1,2], K. Sruthi[1] and L. V. Subba Rao[1]

[1]Department of Plant Breeding, ICAR -Indian Institute of Rice Research, Rajendranagar, Hyderabad, 500 030, India.
[2]Department of Rice, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore 641 003, India.
[3]Department of Plant Biotechnology, Centre for Plant Molecular Biology & Biotechnology, Tamil Nadu Agricultural University, Coimbatore 641 003, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author SRC designed the study, performed the statistical analysis, wrote the protocol and wrote the draft of the manuscript. Authors CRAK, SR and MR conceptualized the study. Authors LVSR, GP, JAK, BJ, BD and KS managed the literature searches and editing. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2020/v32i3831011
Editor(s):
(1) Dr. Syed A. A. Rizvi, Nova Southeastern University, USA.
Reviewers:
(1) Rukhshan Khurshid, Shalamar Medical and Dental College, Pakistan.
(2) Shatha Abdul Wadood Al-Shammaree, University of Baghdad, Iraq.
Complete Peer review History: http://www.sdiarticle4.com/review-history/63773

Original Research Article

Received 10 October 2020
Accepted 14 December 2020
Published 01 January 2021

ABSTRACT

Background: Indigenous traditional coloured rices are rich in dietary fibre, resistant starch, minerals, bioactive compounds and antioxidants like anthocyanins, luteins and phenols. Kavuni is one such brownish black medical landrace of rice considered as nutrition supplement since 400BC as it cures gastritis, peptic ulcer and also enhances blood circulation and known for its anti diabetic and anti-inflammatory properties. Lutein is the only dietary oxycarotenoid found in both the macula and lens of the human eye, and acts as blocker of blue light damage, quench reactive oxygen species, prevent age related macular degeneration, cataracts, cardiovascular disease and lung cancer.

**Corresponding author: E-mail: suvarnarani90@gmail.com;*

Aim: The objective of the present investigation was to characterize the lutein content and antioxidant potential of Kavuni and released mega varieties of rice (ASD 16, Swarna Sub1) and its derivatives obtained from crosses ASD 16 and Kavuni; Swarna Sub1 and Kavuni.
Methodology & Results: It was found that lutein (quantified by HPLC) was much higher in Kavuni (225 µg/100 g) compared with white rice varieties (ASD 16-15 µg/100g and Swarna Sub1-21 µg/100 g) and the DPPH (2, 2-diphenyl-1-picrylhydrazyl) scavenging ability (for quantification of antioxidant potential) were in the order (Kavuni >>ASD16, Swarna Sub1. Pigmented grain genotypes having higher lutein content had higher percentage of free radical scavenging activity of DPPH and lower IC_{50} values compared to non pigmented genotypes.
Conclusion: It is conceivable that the medicinal landraces of rice could be exploited as one of the potential sources for plant - based pharmaceutical products.

Keywords: Kavuni; lutein; antioxidant activity; HPLC (High performance liquid chromatography); DPPH (2, 2-diphenyl-1-picrylhydrazyl.

1. INTRODUCTION

Antioxidants are the bioactive compounds which are vital for defending the cells/ body from the free radicals damage. The unpaired electrons in free radicals are formed due to oxidation as a process of normal metabolic activities in every day exposure to the environment. Reactive oxygen species (ROS) or free radicals when produced in excess amount overwhelming the scavenging ability of endogenous antioxidants like enzymes superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase [1], the excess free radicals seek stability through electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells resulting the induction of lipid per-oxidation and mutation in DNA which leads to many human sufferings like cardiovascular and pulmonary diseases, cataracts, immune/autoimmune diseases, inflammation, arthritis, atherosclerosis and brain dysfunction (Parkinson's, Alzhelmer's, Huntington's diseases) [2]. Due to the adverse side effects of synthetic antioxidants leading to carcinogenicity their use in many countries has been tightly supervised as reported by Pujimulyani, 2003. Therefore search for effective and natural antioxidants has become crucial, investigation of chemically active compounds of plants is of urgent need. Lutein, a dihydroxy xanthophyll, is the predominant plant carotenoid distinguished from other carotenoid compounds based on the chemical composition of hydroxyl group attachments to their structures and presence of β-ionone ring and a ε-ionone ring (Fig. 1). It cannot be synthesized *denovo* and must be acquired from dietary food rich in lutein like green leafy vegetables, fruits and eggs and it is the major component of the macular pigment of the retina. The macula lutea or "yellow spot" in the retina is responsible for the central vision and visual acuity.

Lutein is one of the major therapeutic compounds among 600 naturally occurring carotenoids and is the only carotenoids found in both the macula and lens of the human eye, and acts as blocker of blue light damage, quencher of oxygen free radicals and prevent age related macular degeneration and cataracts [4] and hence these macular pigments are considered as own set of 'Polarized Internal Sunglasses'. In addition to playing pivotal roles in ocular health,

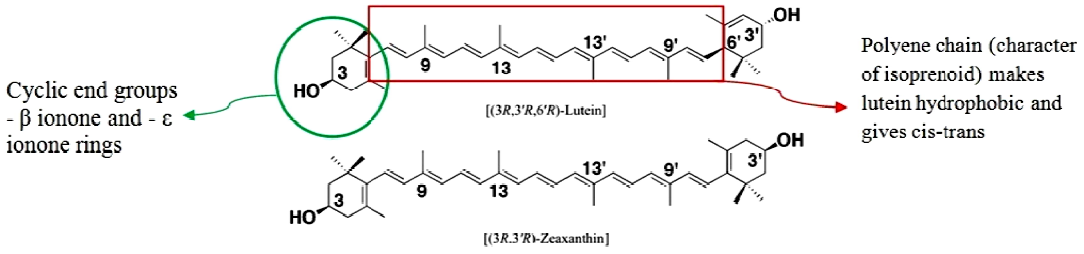

Fig. 1. Chemical structure of lutein and its isomeric forms [3]

lutein and zeaxanthin (isomer of lutein) are important nutrients for the prevention of cardiovascular disease, stroke, and lung cancer. They may also be protective in skin conditions attributed to excessive ultraviolet (UV) light exposure [5]. Coloured fruits and vegetables have higher antioxidants like lutein, than the cereal grains [6], Optimum quantity of coloured fruits and vegetables can't be in the diet of common man. So, an alternate staple food should quench the needs.

Rice is a staple food for more than three billion people in the world. India has been endowed with more than two lakhs rice varieties a rich biodiversity that no other country on earth. The ayurvedic treatise records show the existence of several medicinal rice varieties in India [2]. Kavuni, a traditional rice variety of Tamil Nadu is known for its nutritive properties and it is considered to be the best Antioxidant, Anti-arthritic and Anti-diabetic among other rice varieties (Fig. 2). Kavuni grains are brown-black in colour which were reported earlier to have reduced levels of total soluble sugar, low fat content, increased protein content, high levels of phenolic acid, flavonoids, carotenoids and minerals like iron, manganese, zinc, copper, sodium, potassium, magnesium [7,8]. Kavuni is known as a long duration, poor tillering and photosensitive traditional rice variety and owing to these traits it is not being cultivated widely.

Fig. 2. Husked and Dehusked grains of Kavuni

In our study, crosses were made with major high yield yielding white rice ASD 16 and Swarna Sub 1 to bring out threpeitic rice with good agronomical features. The present investigation reports the lutein content and the antioxidant potential of Kavuni in comparison with ASD 16 and Swarna Sub 1 and its derivatives (Fig. 3). In this research, the pharmacologic potency of the dark coloured Kavuni kernels or grains was partly elucidated with the main objective to find novel source of natural antioxidant to replace the use of synthetic antioxidant.

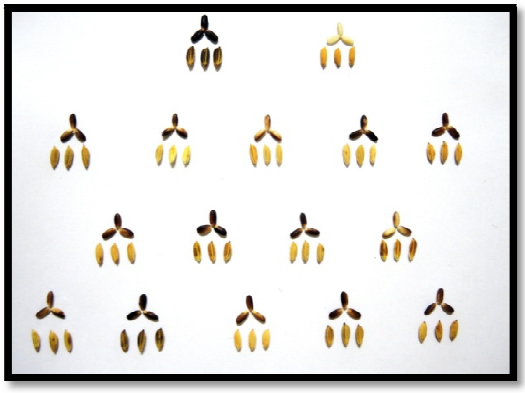

Fig. 3. Seed colour gradiation F_2 derivatives of Swarna Sub1 x Kavuni

2. MATERIALS AND METHODS

The experimental material primarily consisted of two varieties (ASD 16, the popular variety of Tamil Nadu and the mega variety, Swarna Sub1) and one lutein rich landrace, Kavuni obtained from Paddy Breeding Station, TNAU, Coimbatore and the study was conducted at the foresaid place. In this study, Lutein content in the unpolished grains of coloured rice lines was determined by extracting the total carotenoids as described by Lamberts and Delcour [9] and quantified after separation through HPLC as described by Tan and coworkers [10]

2.1 Quantification of Lutein through HPLC Separation

The filtrate was analyzed for quantifying the Lutein content using a Shimadzu HPLC system with LC8A pump, a DAD (190-800nm) UV Vis detector. Separation was carried out in a Phenomenex Develosil 5 µm 140 Å C18 (250 x 4.6mm) using an isocratic elution with acetonitrile (70%), equal volume of methanol and ethyl acetate (30%). The solvents were well sonicated before immersing the rods connected with LC8A pump A and B. Flow rate was set as 1.0 ml/min. A 20 µl volume of the 1.0 ml filtrate was injected into HPLC. Identification and quantification of carotenoids was based on the combined analysis of the retention times, co-chromatography with pure standards and the visible absorption spectra obtained by the photodiode array (PDA) detector. Standard curve was prepared based on the separation of lutein standard injected at various concentrations dissolved in 1.0 ml sonicated methanol. The concentration of lutein in the sample was determined by extrapolating the

standard curve prepared using lutein- range 1.0 µg to 10.0 mg. The standard graph was drawn by plotting concentration of lutein in X-axis and Peak area at Y-axis. Lutein peak was detected at 446 nm. All determinations were performed in triplicate and the reliable peak area obtained in the grain samples was considered for quantification. From the standard graph, the amount of lutein was calculated. The Lutein (carotenoid) content present in the sample is expressed as µg/100 g.

2.2 Estimation of Antioxidant Activity

The antioxidant activity of rice grain samples was estimated by determination of DPPH Radical-Scavenging Activity [11]. The seeds were dehusked using lab dehusker and 1 g of each sample was powdered and extracted with methanol. The solution was evaporated using water bath and the residue was dissolved in 100 ml distilled water. From this 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the extract were pipetted out and made up to 1 ml with methanol, and then 2.5 ml of 0.5 mM methanolic solution of DPPH was added to all the test tubes. The mixture was shaken vigorously and incubated for 37 min in the dark at room temperature. The absorbance was determined at 517 nm using UV visible spectrophotometer (Elico Mini Spec SL171). DPPH free radical scavenging ability was calculated using the formula

$$\text{DPPH radical scavenging activity (\%)} = \frac{[(Ao - As)]}{Ao} \times 100$$

(Antioxidant activity)

Where,

Ao – Absorbance of control
As – Absorbance of sample

The IC_{50} values were calculated using the software GraphPad Prism which is scientific 2D graphing and statistics software published by GraphPad Software Inc., California.

3. RESULTS AND DISCUSSION

3.1 Evaluation of Selected Lines for Lutein Content

Bioavailability of essential lipophilic micronutrients and carotenoids is of utmost interest for human health, as the consumption of these compounds may help alleviate major nutritional deficiencies, prevent cardiovascular disease and cancer. High performance liquid chromatography was used for the quantitative analysis of lutein. Saponification of the samples has been done with 0.5M KOH to get free form of lutein without esters bounded to hydroxyl groups of lutein [12].

High amounts of lutein have been found in Kavuni (225 µg/100g), while both the parents ASD 16 and Swarna Sub1 possess ten times lower the value than that of Kavuni (Fig. 4). Among the coloured and non coloured segregants of F_2 population of both crosses (ASD 16 x Kavuni and Swarna Sub1 x Kavuni), coloured genotypes had comparatively higher levels of lutein than the white grained samples. The findings were in agreement with that of the following studies. Pigmented rice are studied as a major source of antioxidants and other vital functional properties especially lutein and phenolic compounds [13]. Kavuni had higher levels of lutein and antidiabetic in nature [7]. Pereria reported that in rice, high levels of lutein has been reported in several coloured rice varieties in the range of 1.6-2.4 µg/g, whereas lutein content in non-rice cereals such as wheat have been reported to be 4.2 µg/g [14]. Lamberts and Delcour [9], has reported lutein (0.1 µg/g) as the predominant carotenoid present in the bran of brown rice varieties.

3.2 Antioxidant Assay

Antioxidants are vital substances, which possess the ability to protect the body from free radical induced-oxidative stress. Assessments of antioxidant properties of natural compounds are very important because of their uses in medicine, food and cosmetics [15,16,17]. DPPH radical scavenging assay is considered a good in vitro model widely used to assess antioxidant efficacy within short time [2]. Antioxidant activity was measured in terms of IC_{50} value and scavenging per cent of DPPH radicals. IC_{50} value is determined by the sample concentration needed to reduce DPPH activity by 50%. It implies that the lower the value of IC_{50}, stronger is the antioxidant activity of a sample. Kinetic studies of DPPH extract reaction were carried out to estimate scavenging activity as a function of time and sample concentration. Scavenging activity was nearly same at first minute of reaction and diverged with increase of time over a reaction period of 10 minutes and in a concentration dependent manner, the violet colour DPPH solution is reduced to yellow colour

product, by an antioxidant compound to become a stable diamagnetic molecule diphenylpicryl hydrazine.

Free radical scavenging ability of methanolic extract of unpolished grains of parental genotypes *viz*., Kavuni, ASD 16 and Swarna Sub1, had wide range of variation. Purple coloured Kavuni recorded highest values. Among the F_2 segregants of ASD 16 x Kavuni and Swarna Sub1 x Kavuni, ten coloured and ten non-coloured samples each were selected based on colour gradiation, DPPH radical quenching ability was more for the coloured genotypes in both the crosses. The samples were assessed in triplicates and at five different concentrations and the average percentage of DPPH scavenging activity were calculated with ascorbic acid as a standard reference compound and methanol as a blank used in the assay.

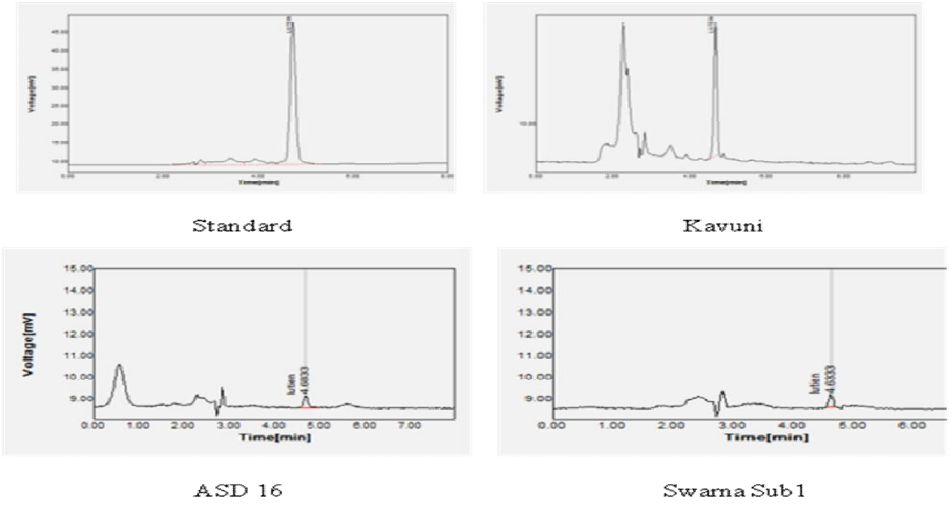

Fig. 4. HPLC chromotogram showing seperation of lutein for rice genotypes

Table 1. DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging assay

Treatments	Replication	DPPH scavenging % at different concentrations					IC_{50} value	SE
		50	100	200	400	800		
Methanol	R1	3.52	7.87	10.01	13.92	16.24	41.05	0.033
	R2	3.45	7.75	9.90	14.49	17.70		
	R3	3.28	7.91	10.06	14.72	17.28		
	Mean	3.41	7.84	9.99	14.38	17.07		
Ascorbic acid	R1	84.92	87.17	86.91	93.48	98.97	0.08	0.197
	R2	83.17	87.82	87.57	91.73	98.14		
	R3	78.78	85.54	87.83	94.18	96.06		
	Mean	82.29	86.85	87.44	93.13	97.72		
Kavuni	R1	48.16	58.23	74.73	86.83	88.05	0.59	0.036
	R2	50.29	57.92	71.75	86.08	92.16		
	R3	48.77	58.14	70.55	86.62	86.40		
	Mean	49.07	58.09	72.34	86.51	88.87		
ASD 16	R1	16.84	22.90	27.78	32.33	33.06	8.02	0.025
	R2	16.49	24.17	27.48	30.21	33.64		
	R3	15.64	22.39	26.72	30.82	33.23		
	Mean	16.32	23.15	27.33	31.12	33.31		
Swarna Sub1	R1	19.78	22.39	25.31	32.22	33.09	11.21	0.091
	R2	20.73	23.34	25.35	31.35	35.79		
	R3	20.46	23.73	25.32	31.01	33.86		
	Mean	20.32	23.15	25.33	31.53	34.25		

Fig. 5. DPPH scavenging assay of ASD 16, Kavuni and ASD 16 x Kavuni derivatives

3.2.1 Free radical scavenging assay for DPPH by parents and F_2 segregants

The DPPH scavenging per cent was 88.87% of methanolic extracts of Kavuni, while it was 33.31% in ASD 16 and 34.25% in Swarna Sub1, while the IC_{50} value is 0.59 for Kavuni and 8.02 µg/ml and 11.21 µg/ml for ASD 16 and Swarna Sub1 respectively. Ascorbic acid which is a positive control had high free radical scavenging per cent of 97.72 % and IC_{50} value of 0.08 µg/ml (Table 1).

In cross 1(ASD 16 X Kavuni) among the coloured and non coloured genotypes, the DPPH scavenging percent and IC_{50} value had great variability. It ranged from plant no. A18 (21.22%, 81.89 µg/ml) to plant no. A20 (40.37%, 6.11 µg/ml) for DPPH scavenging percent and IC_{50} value respectively, while the coloured genotypes had higher value for plant no. A6 (70.64%, 9.72 µg/ml) and lower value for plant no. A5 (47.91%, 9.72) for DPPH scavenging per cent and IC_{50} value respectively (Fig. 5).

In cross 2 (Swarna Sub1 x Kavuni), IC_{50} value ranged from 6.02 µg/ml to 69.77 µg/ml for plant no. S16 and plant no. S12 respectively among the non coloured segregants, while the lower DPPH scavenging per cent and IC_{50} value (68.98% and 1.01 µg/ml) recorded for plant no. S7 and higher value for plant no. S9 with DPPH scavenging per cent (94.31 %) and IC_{50} value (0.22 µg/ml).

On comparing the IC_{50} values and DPPH scavenging per cent of parental material, Kavuni had much higher values for DPPH scavenging per cent and lower IC_{50} values than both the female parents, on the other hand, the coloured segregants of two crosses also had higher values than the respective non coloured genotypes, but lesser than Kavuni.

The IC_{50} values and the scavenging ability were in the order (Ascorbic acid > Kavuni >>ASD16, Swarna Sub1). The findings were also supported by the previous reports of Akiri S Rao,Yodmanee and Valarmathi [6,7,18].

The DPPH scavenging activity of the parental genotypes (ASD 16, Swarna Sub1 and Kavuni), derived and selected genotypes from both the crosses were quantified for lutein content.

Genotypes having higher lutein content had higher percentage of free radical scavenging activity of DPPH and lower IC_{50} values and exhibiting higher antioxidant potential. The IC_{50} values which indicates neutralizing 50% of free radicals of DPPH by the sample concentration and attaining IC_{50} values at lower concentration indicates higher antioxidant activity. Previous literature findings of Saha [18]; Mishra and Sumanta [19] and Krishnanunni [20] supported this study.

4. CONCLUSION

The phytochemical and in vitro antioxidant analysis confirms the methanolic extracts of Kavuni kernels as a potent antioxidant. Kavuni

had broad spectrum in treating the health alignments due to rich source of bioactive molecules like lutein and it is a highly valuable source of natural antioxidants and free radical scavengers. Kavuni is a good choice for the plant breeders to develop new rice cultivars with high nutritive value along with a good yield which can lead to low cost production of natural antioxidants and can reach the bowl of common man.

CONSENT

It is not applicable.

COMPLIANCE WITH ETHICS REQUIREMENTS

- Authors state research work is in compliance with ethics requirements
- This article does not contain any studies with human or animal subjects.

ACKNOWLEDGEMENTS

Authors sincerely acknowledge TNAU, Coimbatore for providing facilities for the conduct of research and DST (Department of Science and Technology (DST) - Innovation in Science Pursuit for Inspired Research (INSPIRE) for providing funding.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sanmugapriya E, Venkataraman S. Studies on hepatoprotective and antioxidant actions of Strychnos potatorum Linn. Seeds on CCl4-induced acute hepatic injury in experimental rats. J. Ethnopharmacol. 2006;105(1–2):154–160.
2. Suvarna Rani Ch, Mini ML. Studies on Antioxidant Activity in Medicinal Land Races of Rice. Indian J. Appl. Res. 2013;3(12):8-9.
3. Delgado-Vargas F, Octavio Paredes-Lopez. Effects of enzymatic treatments of marigold flowers on lutein isomeric profiles. J. Agric. Food Chem. 1997;1(45): 1097-1102.
4. Landrum JT, Bone RA. Lutein, zeaxanthin, and the macular pigment. Arch. Biochem. Biophys. 2001;385:28–40.
5. Yeum KJ, Russell RM. Carotenoid bioavailability and bioconversion. Ann. Rev. Nutr. 2002;22:483-504.
6. Rao AS, Reddy SG, Babu PP, Reddy AR. The antioxidant and antiproliferative activities of methanolic extracts from Njavara rice bran. BMC Complement Altern Med. 2010;28:10(1):4.
7. Valarmathi R, Raveendran M, Robin S, Senthil N. Unraveling the nutritional and therapeutic properties of 'Kavuni' a traditional rice variety of Tamil Nadu. J. Plant Biochem. Biotechnol. 2015;24(3): 305-315.
8. Hemamalini S, Umamaheswari S, Lavanya R, Umamaheswara Reddy C. Exploring the therapeutic potential and nutritional properties of 'karuppu kavuni' variety rice of Tamil nadu. Int J Pharma Bio Sci. 2018; 9(1):88-96.
9. Lamberts L, Delcour JA. Carotenoids in raw and parboiled brown and milled rice. J. Agric. Food Chem. 2008;56:11914-11919.
10. Tan J, Baisakh N, Oliva N, Parkhi V, Rai M, Torrizo L, Datta K, Datta SK. The screening of rice germplasm, including those transgenic rice lines which accumulate beta-carotene in their polished seeds, for their carotenoid profile. Internat. J. Food Sci. Technol. 2005; 40:563-569.
11. Blois MS. Antioxidant determinations by the use of stable free radical. Nature. 1958;181:1199-1200.
12. Li L, Chen CY, Aldini G, Johnson EJ, Rasmussen H, Yoshida Y, Niki E, Blumberg JB, Rusell RM, Yeum KJ. Supplementation with lutein or lutein plus green tea extracts does not change oxidative stress in adequately nourished older adults. J Nutr Biochem. 2010;21(6): 544-549.
13. Frei M, Becker K. Fatty acids and all-trans-beta-carotene are correlated in differently colored rice landraces. J Sci Food Agric. 2005;85:2380-2384.
14. Pereira-Caro G, Cros G, Yokota T, Crozier A. Phytochemical profiles of black, red, brown, and white rice from the camargue region of France. J Agric Food Chem. 2013;61:7976-7986.
15. Halliwell B. Antioxidants in human health and disease. Ann Rev Nutr. 1997; 16:33–50.
16. Sanchez-Moreno C. Methods used to evaluate the free radical scavenging

activity in foods and biological systems. Food Sci Technol Internat. 2002;8(3):121–137.
17. Liu RH, Eberhardt MV, Lee CY. Antioxidant and antiproliferative activites of selected New York apple cultivars N Y Fruit Q. 2001;9:15-17.
18. Saha P, Thamil Selvan V, Kumar Mondal S, Mazumder UK, Gupta M. Antidiabetic and antioxidant activity of Methanol extract of Ipomoea reptans Poir aerial parts in Streptozotocin induced diabetic rats. Pharmacology Online. 2008;1:409-421.
19. Mishra P, Sumanta L. Oxidative stress and heart failure in altered thyroid states Sci World J. 2012;1-17.
20. Krishnanunni K, Senthilvel P, Ramaiah S, Anbarasu A. Study of chemical composition and volatile compounds along with in-vitro assay of antioxidant activity of two medicinal rice varieties: Karungkuravai and Mappilai samba. J Food Sci Technol. 2014;52(5):2572-84.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/63773