Available online at www.journalijmrr.com

INTERNATIONAL JOURNAL OF MODERN RESEARCH AND REVIEWS

Int. J. Modn. Res. Revs.

Volume 5, Issue 1, pp 1450-1456,January, 2017

ISSN: 2347-8314

ORIGINAL ARTICLE

COMPARATIVE EFFECT OF pH AND EXTRACTION TIME ON ANTIOXIDANT PROPERTIES OF TAMIL NADU AND THAILAND RICE VARIETIES

B. Jayanthi and *P. Indra Arulselvi

Plant and microbial Biotechnology Laboratory, Department of Biotechnology, Periyar University, Periyar Palkalai Nagar, Salem, Tamil Nadu-636 011. India

Article History: *Received 5th December,2016, Accepted 31st December,2016, Published 1st January,2017*

ABSTRACT

Rice (*Oryza sativa L.*) is the major cereal and staple food crop provides energy for 50% of the world population. The aim of this study is to evaluate the total phenolics, monomeric anthocyanin, DPPH radical scavenging ability, antioxidant of Tamil Nadu black Kavuni rice (BKR) of Tamil Nadu and Thailand red rice (TRR) with respect to pH and extraction time. Phytochemical screening was also done using FTIR and GC-MS analyses. BKR showed the highest total phenolics 63.7±4.4µg of GAE/g of flour than TRR at pH 6.8 on 2 hours extraction time. Monomeric anthocyanin content of BKR was 1021±97 µg/g flour, which significantly higher than TRR at pH 6.8 on 4 hours extraction time. The stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging ability found to be better in BKR variety, which had IC_{50} value 34.57±0.3g flour /g DPPH at pH 6.8 on 2 hours. Total antioxidant activity found to be higher in BKR (18.6±0 µM AAE/g flour) at pH 6.8 on 4 hours extraction time. FTIR confirmed the functional groups of the phytochemicals present and further verified with GC-MS. Phytochemical screening using GC-MS revealed the presence of industrially important food flavoring agents like tetra, octadecanoic acids and vasodilator, Z,Z-6,28-Heptatriactontadien-2-One.

Keywords: Total Phenolics, DPPH, monomeric anthocyanins, antioxidant and GC-MS

1.INTRODUCTION

Rice (*Oryza sativa L.*) is the staple food crop consumed by more than half of the world's population, mainly in Asia and Africa. It is rich in nutrients and contains a number of vitamins and minerals (www.irri.org/rice basics). Since the white rice is mostly consumed by people, coloured rices also being part of their diet. Coloured rices are potential source of antioxidants, which are in use from long back in history. Aleurone, pericarp and seed coat of the rice grains, which possess most of the antioxidant properties and anthocyanin pigments (Chaudhary, 2003). Red rices are popular in Japan, as it contains high amount of polyphenols and anthocyanin pigments (Itani and Ogawa, 2004). Likewise, Black rice also gained more importance over the white rice cultivar, due to its high anthocyanin content (Suzuki *et al*., 2004).

Research studies on Anthocyanin rich pigmented rice extracts confirmed/demonstrated enhanced atherosclerotic plaque stabilization in apolipoprotein E-Deficient mice (Xiaodong *et al*.,2006), exerted inhibitory effect of cell invasion on various cancer cells (Chen *et al*., 2006) , highly effective in reducing cholesterol levels in the human body (Lee *et al*., 2008), antimutagenic and anticarcinogenic activities (Hyun & Chung, 2004; Nam *et al*., 2005),aldose reductase inhibitory activity (Yawadio *et al*., 2007) and attenuation of some metabolic abnormalities associated with high fructose diets, including glucose intolerance and hyperlipidemia (Guo *et al*., 2007). Antioxidant activities and anthocyanin profiling of rice and rice bran were studied by many researches (Sompong *et al*., 2011).

To study the phenolic antioxidants from the rice or rice bran and its extracts, solvent extraction is commonly used by the researchers, due to simple methodology and effectively low cost. The extraction of phenolic compounds from the starchy rice and rice bran depends upon various parameters viz., polarity and pH of the solvent, solvent- water ratio, temperature, structure and stability of phenolic compounds and anthocyanin in that particular solvent and extraction time (Awika *et al*., 2004; Pe´ rez-Jime´nez & Saura-Calixto, 2006; Sun & Ho, 2005). Hence, it is essential to uncover the systematic conditions to get the higher amounts of phenolics, antioxidants and anthocyanins from the crude extracts. Still today, no one has reported antioxidant activity and anthocyanin profiling in traditional landraces of Tamil Nadu, especially optimized condition for solvent extraction. Moreover, there is no comparison of our landraces with commercially available Thailand rices, based on effect of pH and extraction time with specific solvent. However, most studies on antioxidant activity of pigmented rice extract did

*Corresponding author: ***Dr.P. Indra Arulselvi,*** *Plant and microbial Biotechnology Laboratory, Department of Biotechnology,Periyar University, Periyar Palkalai Nagar, Salem, Tamil Nadu-636 011. India*

not report details on the optimization of the solvent extraction process. Hence, we had compared the effect of two different pH and extraction time on antioxidant properties of Black Kavuni rice and Thailand red rice.

2.MATERIALS AND METHODS

Materials and Sample Processing

Black Kavuni rice (BKR) from Tamil Nadu and Red rice from Thailand was used in this study. Kavuni rice was obtained from Federation for Integrated Improvement in Agriculture Education (FIARE) shop, Attur, Salem District, Tamil Nadu. Thailand red rice (TRR) varieties were purchased from a supermarket in Singapore. Rice flour for analysis was prepared shortly before analysis and passed through the sieve of size 0.25mm. The whole kernels and the rice flour samples were kept at 4°C prior to analysis in polypropylene bags (100µm thickness). All experiments were done at least in triplicates and analytical results were expressed on the basis of dry matter.

Preparation of crude extract

BKR and TRR flours were extracted with aqueous acetone (70:30 v/v, acetone: water). The solvent pH was varied to 2 (acidic) and 6.8 (neutral). 10 g of the rice flour was mixed with 100 ml solvent. The extraction was done twice for equal period of time in each round to acquire the total extraction time of 2 and 4 h. All extractions were done at 32 °C in a shaking water bath. After each round of the extraction, the supernatant and pellet was separated by centrifugation at 1250 ×g for 15 min at room temperature. The supernatant from first and second round of extraction was mixed and evaporated (Tananuwong and Tewaruth, 2010). The final volume of the crude extract was adjusted to 40 ml, stored in brown glass bottles at −18 °C until use. The extraction was done thrice for both varieties.

Determination of total phenolic content

Total phenolic content was measured by Folin- Ciocalteu assay method (Waterhouse, 2005). 1 ml sample was added into a 100 ml volumetric flask, followed by 70 ml of distilled water and 5 ml of Folin-Ciocalteu reagent. The solution was tapped to mix and incubated for 7 min at room temperature. Fifteen ml of sodium carbonate solution was added immediately and made up to final volume with distilled water. The solution was mixed and incubated at room temperature for 2 h. The absorbance was measured at 765 nm by Systronics Double Beam spectrophotometer 2203. Gallic acid was used to make standard curve, the results were expressed as mg gallic acid equivalents/g flour.

Determination of total monomeric anthocyanins

Differential pH method was used to determine the total monomeric anthocyanin content (Giusti &Wrolstad, 2005). The crude extracts were diluted 20 times with pH 1.0 potassium chloride buffer and pH 4.5 sodium acetate buffers, respectively. The maximum absorption of the sample in pH 1 buffer was measured at 513 nm, which indicated that the major anthocyanin in the extract was likely to be cyanidin-3-glucoside. Therefore, the total monomeric anthocyanin content of crude extract was calculated in terms of cyanidin-3-glucoside. The concentration of monomeric anthocyanin pigment was calculated by the following equation:

Monomeric anthocyanin pigment (mg/l) = [Ab_{diff} ×MW×DF ×1000]/ε

where MW represents molecular weight of cyanidin-3-glucoside (449.2), DF is the dilution factor (20), ε is molar absorptivity of cyanidin-3-glucoside (26,900 l/mol cm) and Abdiff was calculated from the following equation:

$$Ab_{diff} = (A_{513} - A_{700})_{pH1} - (A_{513} - A_{700})_{pH4:5}$$

The Absorbance at 700nm was measured and subtracted off in order to eliminate the effect of haze or sediments in the sample.

DPPH radical scavenging assay

DPPH radical scavenging ability of the rice crude extracts was determined by the method of Brand-Williams, Cuvelier and Berset, 1995. Varied dilutions of the crude extracts were prepared. Fifty µl of the diluted crude extract was made up to 950µl of 4mg/100ml DPPH solution in a cuvette and held for 30 min at room temperature. The absorbance was read at 515 nm. EC50 (g flour/g DPPH) is the concentration of the antioxidant that caused the decrease of DPPH radicals to half of its initial concentration, was determined from the graph of the equivalent amount of the sample in DPPH solution (g flour/g DPPH) and radical scavenging activity (%). The second parameter was calculated from the following equation.

Percent inhibition activity = [(A0 - A1)/A0] ×100

where A0 is the absorbance of the control, and A1 is the absorbance of the crude extract.

FTIR analysis

The finely powdered 2% potassium bromide was mixed with dried rice extracts and grind to a fine powder. The sample should be very finely ground as in the Nujol mulling technique to reduce scattering losses and absorption band distortions. The mixed powder was pressed using hydraulic pump. When the pressure reaches 20,000 prf, left for few seconds and release it. The pelleted sample was inserted into the IR sample holder, attached with tape.

GC-MS analysis

Sample preparation

BKR was selected for GC-MS and NMR analysis, based on the biological activities. Preparation of sample from BKR was done as follows: Briefly, black and red rice powder was extracted twice with ethanol/water/ hydrochloric acid (50:50:0.5, v/v/v) of solid–liquid ratio (1:10) for 2 h at 50 °C. The filtrates were combined and subjected to vacuum evaporation to remove ethanol. The solid precipates were packed in centrifuge tubes for further analysis and stored at -20° C.

Instruments and conditions

GC-MS facility was provided by Sophisticated Instrumentation Facility (SIF), VIT University, Vellore. GC-MS analysis was carried out on Thermo fisher GC-MS TurboMass/ MS Quantum TSQ XLS using DB-5MS (30 m×0.25 mm× 0.25 µm) column with helium as carrier gas. Carrier gas helium was used at a flow rate of 1.0 ml/min. The initial column temperature was maintained at 60 °C, keeping 2 min and then heated to 150 °C with 40 °C/ min, and then to 200 °C with 4 °C/min, and then to 230 °C with 2 °C/min, and then 300 °C with 4 °C/min keeping for 6 min; samples were injected in split injection mode with split ratio of 10:1. The total run time was 30 min. Mass spectra were taken at 70 eV and was operated in scan mode from m/z 50–600Da.

GC-MS analysis

One milliliter of sample in the sample vial was kept in the auto-injector and 1 µl of sample was injected in to the GC column using syringe in split mode. The injection temperature was kept at 290 °C. The sample was injected with split mode using split ratio of 10. Initial column temperature was kept at 60 °C for 2 min, and ramp heated to 150 °C with 10 °C/ min then to 200 °C with 4 °C/min, then to 230 °C with 2 °C/min and then to 300 °C with 4 °C/min keeping for 6 min. A 30 m×0.25 mm×0.25 µm thickness of DB5-MS capillary column 5%phenyl—95%dimethyl polysiloxane cross bonded liquid phase capillary column was used with 99.9995 % helium as carrier gas with a flow rate of 1.0 ml/min in a split mode (10:1). The total GC cycle run time consisted of 30 min. The MS was operated in the scan mode from m/z 50 to 650. Each compound was identified by the presence of selected ions and their ratio, and by comparing the retention index and similarity index in mass spectra of the samples to the reference in the national institute of standards and technology (NIST, ver. 2.0, 2008) mass spectral database.

Statistical Analysis

All the tests were performed in triplicates and values were expressed as mean ± standard deviation. Data were statistically analysed using ANOVA and Tukey's test. Both IC50 and ANOVA were done using Graph Pad Prism Software Version 5.0. $P<0.05$ was considered as statistically significant.

3.RESULTS AND DISCUSSION

Total Phenolic and Monomeric Anthocyanin Content

BKR and TRR crude extracts have better activity in the total phenolic content. BKR showed the highest concentration of total phenolics (63.7±4.4µg of GAE/g of DW), followed by TRR (44.1±2.2µg of GAE/g of DW) at pH 6.8 on 2 hour extraction time. When compared to red rice crude extracts, black rice crude extracts obtained from two different extraction time and pH showed significantly higher concentration of phenolic content by dry weight of the sample. Differences in extraction time have significant effect on total phenolic content (Table.1). Neutral pH played much role in extraction of phenolics in BKR, but not significantly changed the TRR phenolic content. Previous reports by Sompong *et al*., 2011 and Tananuwong and Tewaruth, 2010 also confirmed the similar type of Phenolic content in Black and red rice varieties.

The overall results of the total monomeric anthocyanin contents of the crude extracts obtained from different extraction conditions were shown in Table.1. Statistical analysis showed that extraction time did significantly affected total monomeric anthocyanin content of the crude extract, while same factor significantly influenced total phenolic content of the extract. The crude extracts obtained from longer extraction time tended to have higher amount of monomeric anthocyanins. Acidity of the solvent had no positive impact on total monomeric anthocyanin contents. At the similar extraction duration, neutral solvent (pH 6.8) provided the extract with significantly higher amount of total monomeric anthocyanin than acidic solvents (pH 2). BKR variety had higher monomeric anthocyanin content (1021±97 µg/g flour) than TRR in both pH conditions. It showed highest amount of anthocyanins, especially at pH 6.8 by 4 hours extraction time. Sompong *et al*., 2011 reported that nearly 691.37mg/100 g flour of monomeric anthocyanins in red rice variety. Our BKR variety showed much more anthocyanin content than the above said report.

Total Antioxidant Activity

BKR and TRR varieties showed more total antioxidant activity at pH 6.8 by 4 hour extraction time. Extraction time played an important in determining the total antioxidant content of rice samples. The antioxidant activity followed the order: pH 6.8 (4 hours) extracted samples > pH 6.8 (2 hours) extracted samples > pH 2 (4 hours) extracted samples > pH 2 (2 hours) extracted samples. Extraction time and pH showed its impact on total antioxidant activity and total phenolics differently. When compared to acidic condition, neutral pH worked out well for both the activities, represented in the Table 1. Total antioxidant activity found to be expressed well in pH 6.8 at 4 hour extraction time for BKR (18.6±0 µM AAE/g flour). TRR variety did not show much significant difference throughout both pH and extraction time. BR5 rice variety showed similar TAC (701±1.44µM/100g), when compared to BKR (Dutta *et al*., 2012).

DPPH Radical Scavenging ability

Black Rice Variety BKR showed lowest percentage of DPPH remaining 5.68 % for 50µg/g flour, compared to TRR (fig.1A). The lowest percent of DPPH remaining had the highest anti-oxidant efficiency. The crude extracts obtained from longer extraction time tended to have greater anti-oxidant activities. From the graph (fig.1A&1B), it has been considered that 2 hours extracted samples had lower inhibitory concentration for DPPH (34.57±0.3 g flour /g DPPH). At similar extraction duration, acidic solvent might extract a group of compounds with less DPPH radical-scavenging activities. Neutral pH condition worked well for the radical scavenging activity (fig.1A & 1B). Acidic system took more time to reach steady state (Tananuwong and Tewaruth, 2010). Hence, the lower antioxidant activity of the acidic system from this study could be due to the alteration in antioxidant composition.

Table.1. Total Phenolics (TPC), Total Monomeric anthocyanin (TMA) content and total antioxidant activity (TAA) of Black Kavuni rice and Thailand red rice extracted at different extraction time and Solvent pH

Solvent pH	Total Extraction Time (h)	TPC (µg of GAE/g flour)		TMA content (µg/g flour)		µM AAE/g flour)		(g flour /g DPPH)	
		BKR	**TRR**	**BKR**	**TRR**	**BKR**	**TRR**	**BKR**	**TRR**
2.0	2	38 ± 4.4^{c}	35.3 ± 1^{c}	247 ± 14^{b}	73 ± 47^{c}	12.3 ± 1.5^{c}	15 ± 0.1^{c}	43.76 ± 0.2^{a}	38.66 ± 0.12^{b}
	4	46.6 ± 5.8^{b}	43 ± 1.2^{c}	260 ± 50^{b}	80 ± 15^{c}	14.9 ± 0.1^{c}	15.4 ± 0.1^{c}	41.75 ± 0.3^{a}	37.79 ± 0.5^{b}
6.8	2	63.7 ± 4.4^{a}	44.1 ± 2.2^{c}	541 ± 04^{b}	100 ± 19^{c}	16.4 ± 0.1^{b}	16.4 ± 0^{b}	34.57 ± 0.3^{c}	40.95 ± 0.6^{b}
	4	51 ± 4.4^{b}	42.1 ± 1.2^{c}	1021 ± 97^{a}	127 ± 47^{c}	18.6 ± 0^{a}	17.3 ± 0^{a}	44 ± 0.43^{a}	39.24 ± 0.1^{b}

Data were mean ± standard deviation (n=3), Data were statistically analysed using ANOVA and Tukey's test.
Mean present in the same column with different superscripts are differ significantly ($p<0.05$)

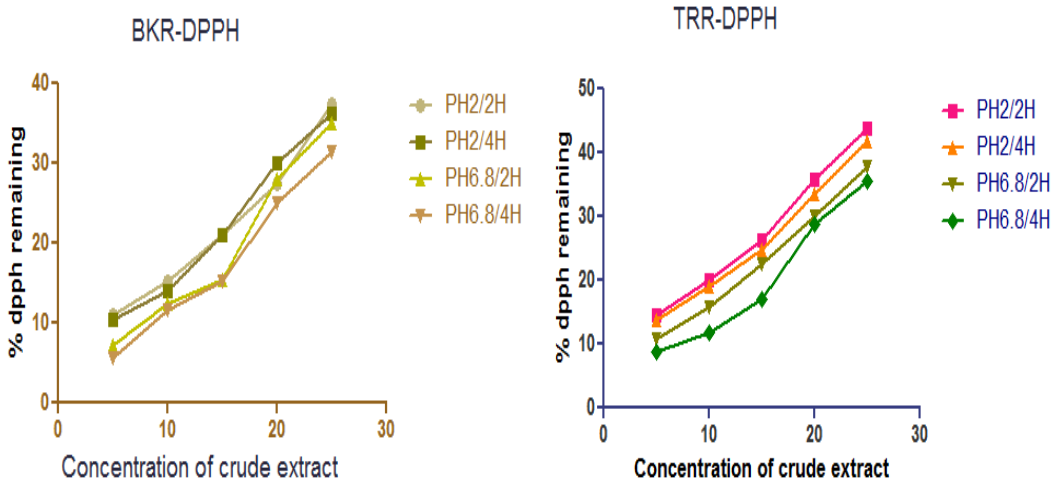

Fig.1. Graphical representation of DPPH radical scavenging activity assay at different dilutions of crude extract. 1A. Line graph of BKR variety, 1B. Line graph of TRR variety, BKR-Black Kavuni Rice and TRR-Thai red rice

Table.2. Biological activity of phytocompounds identified from GC -MS spectrum analysis

S.No.	RT	Name of the compound	Molecular weight	Molecular Formula	Component Activity
1.	18.11	Hexadecanoic Acid	256	$C_{16}H_{32}O_2$	Antioxidant, Hypocholesterolemic, Flavor
2.	18.12	Octadecanoic Acid	284	$C_{18}H_{36}O_2$	Binder in food products, production of margarines, spreads, butter, etc, Antiarthritic, Hepato protective
3.	18.13	Tetradecanoic Acid	228	$C_{14}H_{28}O_2$	Flavor ingredient, food additive
4.	19.76	Z,Z-6,28-Heptatriactontadien-2-One	530	$C_{37}H_{70}O$	Vasodilator
5.	23.88	Adipic Acid	338	$C_{20}H_{34}O_4$ $C_{29}H_{50}O$	Food additive, food ingredient Anti-inflammatory, antipyretic, treating hypercholesterolemia
6.	29.28	beta-Sitosterol	414	$C_{29}H_{50}O$	Anti-inflammatory, antipyretic, treating hypercholesterolemia

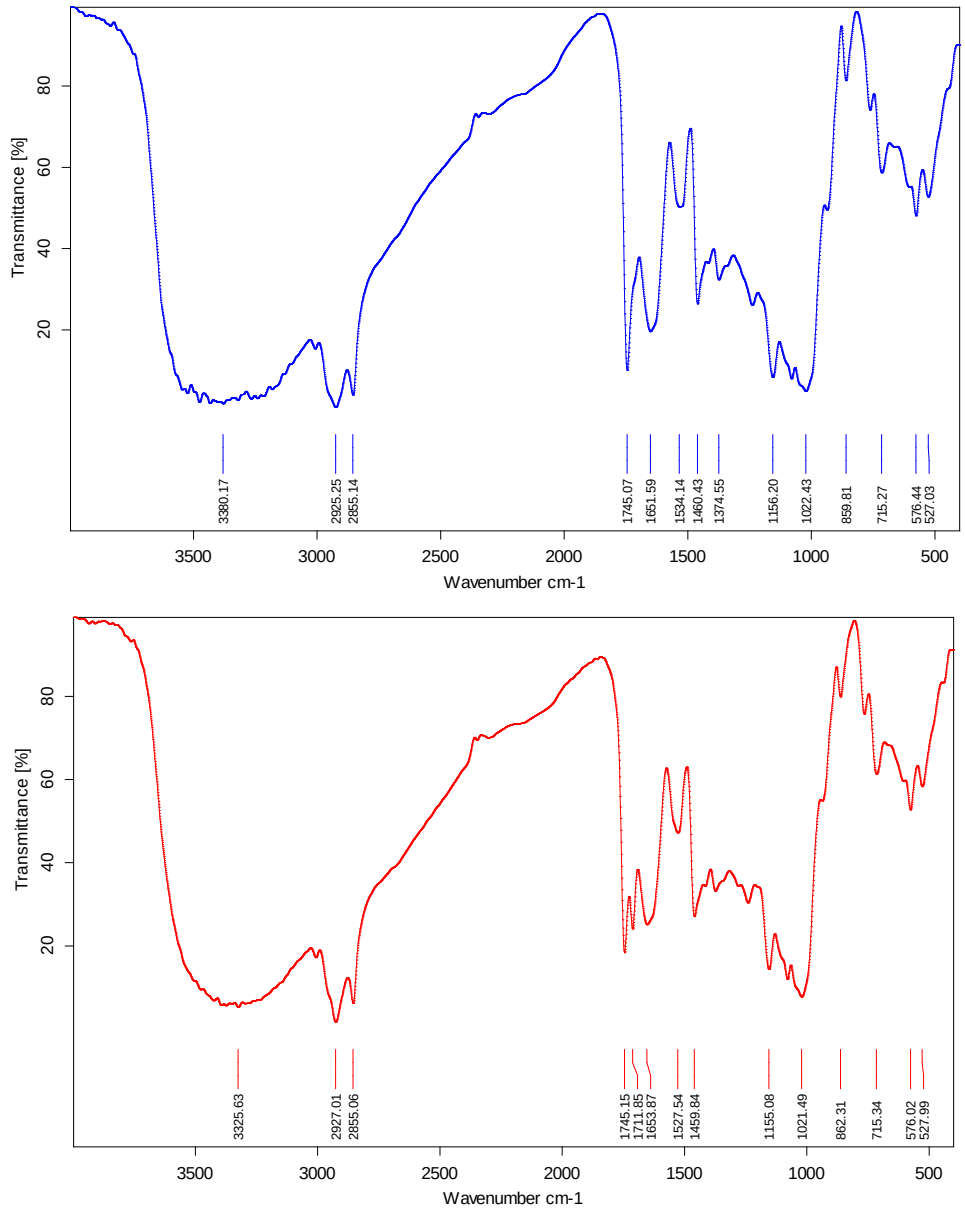

Fig. 2A. IR Spectrum for BKR, 2B. TRR rice varieties. BKR-Black Kavuni Rice, TRR-Thai Red Rice.

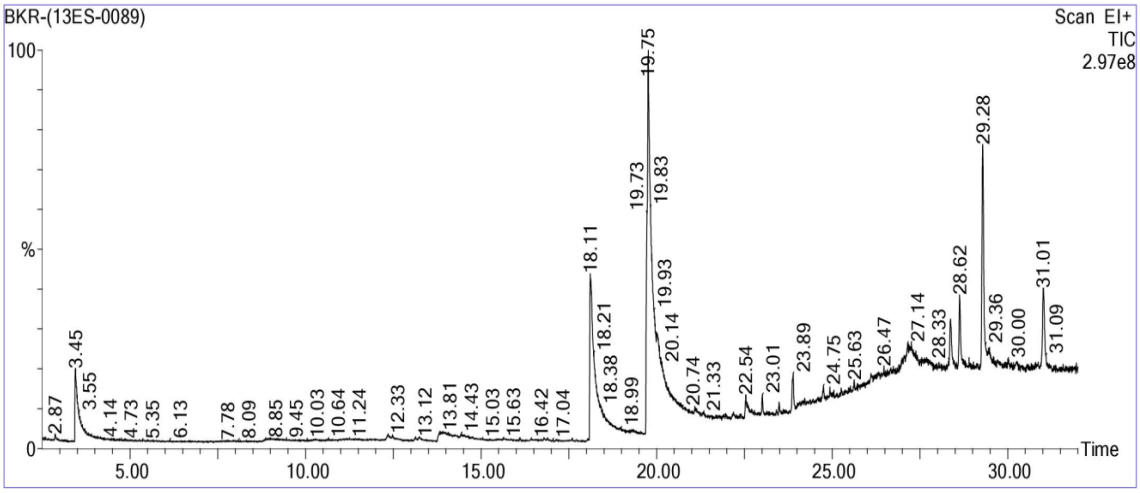

Fig.3. GC-MS analysis of Black Kavuni Rice

FTIR analysis

The IR spectrum of BKR, a very intensely broad band at 3380 cm^{-1} and moderately intense band at 1156.9 and 715 cm^{-1} were observed for the O-H bond vibrations of hydroxyl group. The out of plane C-H vibrations of the unsaturated part was observed at 859cm^{-1}. The corresponding C=C vibrations was shown around 1651cm^{-1} as weakly intense band. The stretching and bending vibrations of methyl part were noticed by the intense band 2925 cm^{-1} and medium intensity band at 1460cm^{-1}. The vibration of the methylenic part was shown by the band at 2856cm^{-1} and the medium band at 1460cm^{-1}.The moderately intense band at 730cm^{-1} was attributed to the rocking movement of methylenic part. The corresponding C-C vibration was shown as weak intense band at 1022cm^{-1}. These functional groups represent the lipid or carboxylic acid functional groups.

Similarly from the IR spectrum of TRR, a very intensely broad band at 3325cm^{-1} and moderately intensely broad band at 1745cm^{-1} and 715cm^{-1} were observed for the O-H bond variations of hydroxyl groups. The out of plane C-H vibrations of the unsaturated part was observed at 862cm^{-1}. The corresponding C-C vibrations were shown around 1653cm^{-1} as weekly intense band. The stretching and bending vibrations of methyl part were noticed by the intense band 2921cm^{-1} and medium intensity band at 1459cm^{-1}. The vibration of the methylenic part was shown by the band at 2855cm^{-1} and the medium band at 1459cm^{-1}. The moderately intense band at 715cm^{-1} was attributed to the rocking movement of the methylenic part. The corresponding C-C vibration was shown as weak intense band at 1021cm^{-1}. These functional groups represent the carboxylic acid groups.

GC-MS spectrum for Black Kavuni Rice

Six important compounds were identified in Black Kavuni rice extract by GC-MS analysis. The active principle component, Molecular weight (MW), Molecular formula (MF), Retention Time (RT) and their bioactivity are presented in Table 2 and Fig.3 dictates that the predominant compounds are Hexadecanoic Acid , Octadecanoic Acid, Tetradecanoic Acid, Z,Z-6,28-Heptatriactontadien-2-One, Adipic acid, beta-sitosterol., etc. Volatile profiling of BKR showed some most important phytochemicals, which can be used in therapeutics. Out of the phytochemicals identified in the rice varieties, the majority of them belong to the carboxylic acids, lipids and phytosterols (plants sterols). Plant sterols especially beta-sitosterol studied extensively for their anti-inflammatory; antineoplastic, antipyretic, and immunomodulating activities (Careri *et al*., 2006). Krishnanunni *et al*., 2014 also reported about the phytosterols in two medicinal rice varieties, Mapillai Samba and karungukuruvai. Carboxylic acids like Tetra, Hexa, octadecanoic acid are mostly used in food industries as binder and flavouring agents. They have more antioxidant activity, which confirmed from the study by Krishnanunni *et al*., 2014

4.CONCLUSION

Black Kavuni rice is one of the traditional rice varieties in Tamil Nadu. We compared our rice Kavuni with Thai red rice for its antioxidant, Phenolic and monomeric anthocyanin content. Our study proved the importance of solvent pH and extraction time for its biological activities. FTIR and GC-MS confirmed the presence of phytochemicals like beta-sitosterol in Kavuni rice. Findings from our research may lead to optimized conditions for specific rice varieties to extraction the exact phytochemical. This could be first step in food industries to extract particular nutrients and use it as dietary supplements.

5.ACKNOWLEDGEMENTS

BKR was gifted from Mr. S. Senthil Kumar, President Federation for Integrated Improvement in Agriculture Education (FIARE), Attur, Salem. This work was supported by Department of Science and Technology with Inspire Fellowship, Government of India.

6.REFERENCES

Alak Kanti Dutta, Partha Sarathi Gope, Subrata Banik, Sukh Makhnoon, Muhammad Ali Siddiquee, Yearul Kabir. Antioxidant properties of ten high yielding rice varieties of Bangladesh.Asian Pacific Journal of Tropical Biomedicine. 2012; S99-S103.

Awika, J. M., Rooney, L. W., & Waniska, R. D. Anthocyanins from black sorghum and their antioxidant properties. Food Chemistry. 2004; 90, 293–301.

Brand-Williams, W., Cuvelier, M. E., and Berset, C. Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft und-Technologie.1995; 28, 25–30.

Careri M, Elviri L, Mangia A. Liquid chromatography-UV determination and liquid chromatography-atmospheric pressure chemical ionization mass spectrometric characterization of sitosterol and stigmasterol in soybean oil. J Chromatogr A. 2006; 935:249–257.

Chaudhary, R. C. Speciality rices of the world: Effect of WTO and IPR on its production trend and marketing. Journal of Food, Agriculture and Environment. 2003; 1(2), 34–41.

Chen, P.-N., Kuo, W.-H., Chiang, C.-L., Chiou, H.-L., Hsieh, Y.-S., & Chu, S.-C. Black rice anthocyanins inhibit cancer cells invasion via repressions of MMPs and u-PA expression. Chemico-Biological Interactions. 2006; 163(3), 218–229.

Giusti, M and Wrolstad, R. E. Characterization and measurement of anthocyanins by UV-visible spectroscopy. In R. E. Wrolstad, T. E. Acree, E. A. Decker, M. H. Penner, D. S. Reid, S. J. Schwartz, C. F. Shoemaker, D. Smith, & P. Sporns (Eds.), Handbook of food analytical chemistry: Pigments, colorants, flavors, texture, and bioactive food components. Hoboken, New Jersey: John Wiley & Sons; 2005.

Guo, H., Ling, W. H., Wang, Q., Liu, C., Hu, Y., Xia, M. Effect of anthocyanin-rich extract from black rice (Oryza sativa L. indica) on hyperlipidemia and insulin resistance in fructose-fed rats. Plant Foods for Human Nutrition. 2007; 62, 1–6.

Hyun, J. W., & Chung, H. S. Cyanidin and malvidin from Oryza sativa cv. Heungjinjubyeo mediate cytotoxicity against human monocytic leukemia cells by arrest of G(2)/M phase and induction of apoptosis. Journal of Agricultural and Food Chemistry. 2004; 52, 2213–2217.

Itani, T., & Ogawa, M. History and recent trends of red rice in Japan. Japanese Journal of Crop Science. 2004; 73(2), 137–147.

Krishnanunni K, P. Senthilvel, Sudha Ramaiah, Anand Anbarasu. 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), pp 2572-2584.

Lee, J.-C., Kim, J.-D., Hsieh, F.-H., & Eun, J.-B. Production of black rice cake using ground black rice and medium-grain brown rice. International Journal of Food Science and Technology. 2008; 43(6), 1078–1082.

Nam, S. H., Choi, S. P., Kang, M. Y., Koh, J. H., Kozukue, N., & Friedman, M. Antioxidative activities of bran extracts from twenty one pigmented rice cultivars. Food Chemistry. 2006; 94, 613–620.

Pe´ rez-Jime´ nez, J., and Saura-Calixto, F. Effect of solvent and certain food constituents on different antioxidant capacity assays. Food Research International. 2006; 39, 791–800.

Sompong R, S. Siebenhandl-Ehn, G. Linsberger-Martin, E. Berghofer. Physicochemical and antioxidative properties of red and black rice varieties from Thailand, China and Sri Lanka, Food Chem. 2011; 124, 132–140

Sun, T and Ho, C. Antioxidant activities of buckwheat extract. Food Chemistry. 2005; 90, 743–749.

Suzuki, M., Kimura, T., Yamagishi, K., Shinmoto, H., & Yamaki, K. Comparison of mineral contents in 8 cultivars of pigmented brown rice. Nippon Shokuhin Kagaku Kogaku Kaishi. 2004; 51(8), 424–427.

Tananuwong K and Tewaruth W. Extraction and application of antioxidants from black glutinous rice. LWT - Food Science and Technology. 2010; 43, 476–481.

Waterhouse, A. L. Determination of total phenolics. In R. E. Wrolstad, T. E. Acree, E. A. Decker, M. H. Penner, D. S. Reid, S. J. Schwartz, C. F. Shoemaker, D. Smith, & P. Sporns (Eds.), Handbook of food analytical chemistry: Pigments, colorants, flavors, texture, and bioactive food components (pp. 463–470). Hoboken, NJ: John Wiley & Sons. 2005.

Xiaodong Xia, Wenhua Ling, Jing Ma, Min Xia, Mengjun Hou, Qing Wang, Huilian Zhu and Zhihong Tang J. An Anthocyanin-Rich Extract from Black Rice Enhances Atherosclerotic Plaque Stabilization in Apolipoprotein E–Deficient Mice. J. Nutr. 2006; 136: 2220–2225.

Yawadio, R., Tanimori, S., & Morita, N. Identification of phenolic compounds isolated from pigmented rices and their aldose reductase inhibitory activities. Food Chemistry. 2007; 101, 1616–1625.

*****