Biocatalysis and Agricultural Biotechnology 21 (2019) 101351

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: http://www.elsevier.com/locate/bab

Comparison of phytochemicals, antioxidant and hypoglycemic activity of four different Brown rice varieties

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ARTICLE INFO

Keywords:
Antioxidant
Antidiabetic
Bioactive metabolites
Minerals
Traditional rice

ABSTRACT

Our study investigated the nutrient profiles, bioactive metabolites, antioxidant and anti-diabetic properties of traditional rice varieties such as Puzhuthikar (PTS), Elupaipoo Samba (EPS), Valan Samba (VS) and Garudan Samba (GS). The total phenols and flavonoids were significantly higher in PTS followed by GS, EPS and VS. Gas chromatography and Mass spectroscopy (GC-MS) spectra showed therapeutically relevant secondary metabolites present in the rice varieties. Precisely, this is the first report about the metabolites of traditional rice varieties to the best of our knowledge. PTS displayed higher DPPH radical scavenging and total antioxidant activity, whereas, EPS had the significant capacity to reduce ferric ions and superoxide anions. The α-amylase and α-glucosidase inhibitory concentration reveal the effectiveness of PTS and EPS in type-II diabetes management. Overall, the results suggested that EPS and PTS extracts may afford a novel and an alternative source of nutrients, therapeutically relevant metabolites, antioxidants and blood glucose regulators.

1. Introduction

Rice (*Oryza sativa* L.) plant belongs to the grass family *Poaceae* and it is an economically essential staple food crop for all over the world. India ranks second among rice cultivating countries with total area of about 44.6 million hectares which have been disseminated as irrigated (52.6%), rain fed (32.4%), upland (12%) and submerged regions (3%) with a productivity yield of ~1.9 tonnes per hectare (Muthayya et al., 2014). In India, 90% of the population consume rice as their primary food in everyday meal. Among the rice varieties, pigmented rice received much attention due to their nutrient richness and antioxidant properties (Ravichanthiran et al., 2018). Krishnanunni et al. (2015) reported the potential impact of antioxidant activity with higher phytochemicals presence in the pigmented rice varieties (Karungkuruvai, Mapillai samba).

Brown rice is an unpolished grain, coated with germ, bran, and endosperm. Variations exist in their kernel color in traditional rice varieties, which depends on their metabolite profiles (Ghasemzadeh et al., 2018). Whole rice grains contain major secondary metabolites like polyphenols, phenolic acids, tocopherols, flavonoids, anthocyanins, proanthocyanidins, γ-oryzanol and tocotrienols, in turn contributing in health benefits by acting on scavenging reactive oxygen species (ROS) like lipid peroxide, superoxide, hydroxyl free radicals and such process is considered as most effective antioxidants in nature (Goufo and Trindade, 2014; Shao and Bao, 2015).

Previous studies on pigmented rice reported strong association in nutrigenomic implications such as anti-diabetic, anti-cholesterol, cardio-protective and antioxidant activity (Krishnanunni et al., 2015; Valarmathi et al., 2015). Thus, the present study focused on investigating phytochemicals, and anti-diabetic properties of the traditional/pigmented rice varieties of South India (Tamil Nadu). Accordingly, the present study was conducted i) To characterize whole grain metabolites present in four pigmented traditional rice varieties viz., Puzhuthikar (PTS), Elupaipoo (EPS), Valan (VS), and Garudan Samba (GS), ii) To evaluating their antioxidant activities and (iii) To check the hypoglycemic activities of four rice genotypes (inhibition against

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https://doi.org/10.1016/j.bcab.2019.101351
Received 6 August 2019; Received in revised form 7 September 2019; Accepted 17 September 2019
Available online 18 September 2019

α-amylase and *α-glucosidase*).

2. Materials and methods

2.1. Rice samples and reagents

The rice seeds of the pigmented traditional varieties (*Oryza sativa* L.) viz., Puzhuthikar (PTS), Elupaipoo (EPS), Valan (VS), and Garudan Samba (GS) were obtained from FIARE, Salem and a commercial cultivar IR64 was acquired from the Department of Rice, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India. The dehusked rice grains were ground into fine powder and sieved using 0.48 mm mesh and used for phytochemical and anti-diabetic experiments. In this study,2,2-diphenyl-1-picrylhydrazyl (DPPH), *α-amylase* and *α-glucosidase* from Sigma-Aldrich (Steinheim, Germany). Folin–Ciocalteu's phenol reagent (F–C reagent), 2, 4, 6-tri (2-pyridyl)-s-triazine (TPTZ), rutin, gallic acid, nitroblue tetrazolium chloride (NBT), nicotinamide adenine dinucleotide (NAD), phenazine methosulfate (PMS), Na_2CO_3, $AlCl_3$, ammonium molybdate, NaOH, CH3COONa and $FeCl_3$ were acquired from HiMedia (HiMedia Laboratories, India). Potassium ferricyanide (K3[Fe (CN)6]) was purchased from Merck Life Science Private limited (Darmstadt, Germany). Sulphuric, Nitric and perchloric acids, acetone, methanol and all the chemicals used were of analytical grade and were purchased from Sigma-Aldrich (Steinheim, Germany), HiMedia (Mumbai, India), and Merck Life Science (Darmstadt, Germany).

2.2. Mineral analysis

Minerals profile includes Phosphorus (P), Potassium (K), Magnesium (Mg), Calcium (Ca), Iron (Fe), Zinc (Zn), Manganese (Mn), Copper (Cu) of all the five rice varieties were determined according to the method developed by Anuradha et al. (2012). Briefly, 1 g of rice samples were digested with nitric, sulphuric and perchloric acids mixture (5:2:1). The digested samples were analyzed using Inductive Couple Plasma Atomic Emission Spectrometry (ICP- AES, Thermo Electron Corporation, IRIS INTREPID II XSP DUO).

2.3. Extraction and estimation of total phenolics and flavonoids

The whole rice grains were shade dried and pulverized using mortar and pestle. All the rice flour samples were extracted using acidified acetone at 85 °C ± 2 °C (1:4 (w /v)) in s magnetic stirrer for 2 h (Tananuwong and Tewaruth, 2010). Total phenolics was estimated by modified colorimetric method using Folin's Ciocalteau reagent (Singleton et al., 1999). Briefly, 120 μL of flour extract was mixed with 600 μL of Folin's Ciocalteau reagent along with 1.2 mL of distilled water. The reaction mixture was incubated at room temperature (RT) for 2 min, and 960 μL of 1 M sodium carbonate was added to the mixture. Further, the absorbance of the reaction mixture was measured at 760 nm (UV–Vis-1800 double beam spectrophotometer) (Shimadzu Corp, Japan) after 30 min incubation. Gallic acid and acetone were used as positive and negative controls, respectively. The results were mentioned as gallic acid equivalents per gram of flour dry weight using the absorbance values at 765 nm (μg GAE/g flour DW).

The total flavonoids present in the rice extracts of were determined by using the modified colorimetric method using aluminum chloride (Zhishen et al., 1999). The absorbances were measured at 510 nm and the results were expressed as mg rutin equivalents (RUE)/gram flour DW.

2.4. DPPH radical scavenging and ferric reducing antioxidant power (FRAP) assay

DPPH free radicals scavenging was assessed in rice extracts by using method of Soler-Rivas et al. (2000). The total reaction mixture contains 100 μL of DPPH (90 μM methanolic DPPH) with 10 μL of extract at varied concentrations in a 96-well microplate before diluting the mixture with 190 μL methanol. The extract was substituted with methanol for the negative control and ascorbic acid was used as the reference antioxidant. The decrease in the DPPH radical concentration was measured at 517 nm after 30 min incubation at room temperature. The radical scavenging activity was expressed in terms of the sample quantity needed to decrease the initial absorbance by 50% (IC_{50}).

The ferric reducing power of the rice extracts was determined according to method of Benzie and Strain (1996). Two hundred microliters of rice extracts were mixed with 1.3 mL of FRAP reagent (contains 0.3 M sodium acetate, 10 mM 2, 4, 6-tri (2-pyridyl)-s-triazine (TPTZ) dissolved in 40 mM hydrochloric acid and 20 mM ferric chloride). The mixtures were incubated at 37 °C for 30min, and the absorbance was recorded at 595 nm. The IC_{50} concentration, which is defined as the concentration of antioxidants needed to reduce 50% of the initial concentration of ferric radicals is an index used to compare and express the reducing power of bioactive substances.

2.5. Superoxide anion scavenging and phospho-molybdenum assay

The superoxide anion scavenging assay was determined as described by (Robak and Gryglewsk, 1988). The rice extracts (0.1 mL) were mixed with reagent solution containing Nitroblue tetrazolium chloride (NBT-0.5 mL), Nicotinamide adenine dinucleotide (NAD-0.5 mL) and 16 mM Tris buffer (0.5 mL, pH 8). The reaction was initiated by addition of 120 μM phenazine methosulfate (PMS-0.5 mL). Subsequently, the absorbance was measured at 560 nm after incubation for 5 min. The superoxide radical scavenging ability was provided in terms of the amount of sample needed to decrease the initial absorbance by 50% (IC_{50}).

The total antioxidant capacity of rice extracts was evaluated by the phospho-molybdenum reagent (Prieto et al., 1999). 1 mL of rice extracts were added with 1 mL of phospho-molybdenum reagent (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate), and the reaction mixture was incubated in boiling water bath at 95 °C. After 90 min of incubation, the absorbance was read at 695 nm. Ascorbic acid (10 mg/mL dissolved in DMSO) was used as the standard. The phospho-molybdenum reduction potential (PRP) of the studied rice extracts were reported as IC_{50}.

2.6. Alpha-amylase and alpha-glucosidase inhibitoryassays

Alpha-amylase inhibitory effect was determined as described by Jumepaeng et al. (2013). The rice extracts (500 μL were added with 500 μL of sodium phosphate buffer (pH 6.9) containing α-amylase (0.5 mg/mL) and the reaction mixture was incubated at ambient temperature for 10 min. An equal volume of starch solution was added and further incubated at 37 °C for 10 min. The reaction was terminated by the addition of DNS (dinitrosalicylic) reagent (1 mL) and the tubes were placed in boiling water bath (65–70 °C) for 5 min. The absorbance was measured at 540 nm against a reagent blank.

The α-glucosidase inhibitory effect was determined according to the method of Kim et al. (2005). The significant inhibition of α-glucosidase activity in the sample was calculated with IC_{50} values.

2.7. GC-MS analysis

GC-MS analyses of the rice extracts were performed by injecting 1 μL of the samples in split mode (1:10 split ratio) in a GC-MS-QP2010 PLUS model (Shimadzu, Kyoto, Japan). The qualitative and quantitative analyses of the samples were carried out using a 30-MRTX-5 MS and RTX-5MS GC column (Restek Corporation). The subsequent standard GC-MS operating procedure was followed as: the initial program temperature was 70 °C then increased up to 310 °C and held for 5 min, ion temperature: 230 °C and scan range: 40–600 m/z. The results of the components were based on their comparisons of their mass spectra with those

of Wiley library.

2.8. Statistical analysis

Statistical analysis was carried out using the software package SPSS v20 (SPSS Inc., Chicago, IL, USA) and the comparison of averages was based on the analysis of variance (ANOVA) along with Dunnett's test for comparison among the rice varieties for significance level ($p < 0.05$). Correlation analysis was carried out using Pearson's correlation coefficient (r). Graphs were created using Prism v6.01 (Graph Pad Inc., San Diego, CA, USA) and the results were presented as mean ± standard error of replications.

3. Results and discussion

3.1. Minerals and bioactive compounds

Minerals are the most essential nutritional sources for regular metabolic functions and for maintaining balanced diet. Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) results described that traditional pigmented rice would be abundant source of major minerals comprising potassium, calcium, phosphorus, iron, manganese, magnesium, zinc and copper (Table 1). These nutrients are necessary for a healthy human body and are crucial components governing the efficient functioning of body activities. The mineral contents of the tested rice varieties exhibited significant differences ($p \leq 0.05$), except for zinc, manganese and copper which showed less or no significance in comparison to other fundamental minerals. The most abundant mineral (per 100 mg sample) among the rice varieties was phosphorus (47.47–266.5 mg) followed by potassium (21.65–178.4 mg), magnesium (22.67–97.57 mg) and calcium (9.11–46.04 mg) and, the lowest was copper (0.02–0.39 mg). The results indicated that the mineral concentrations were diverse amongst the genotypes which might be greatly influenced by their genetic constituent. Similarly, wide variationsin mineral compositions were reported in aromatic rice varieties (Renuka et al., 2015). Therefore, the traditional pigmented rice varieties are rich in minerals and can be considered as affordable and useful way to mitigate malnutrition and its related health issues. The highest macro- and micro-mineral contents were found in the traditional pigmented rice varieties when compared to IR64 which showed a lower mineral composition. Thus, high yielding commercial rice varieties can be replaced with mineral-dense pigmented traditional rice varieties to decrease the rate of micronutrient deficiency diseases.

3.2. Total phenolic (TPC) and flavonoid content (TFC) analysis

The phenolic content differed significantly ($p < 0.05$) amongst the rice genotypes and the results were obtained as gallic acid equivalents (GAE) as shown in Table 1. The total phenolic content in the pigmented traditional rice flour extracts ranged from 79.13 to 358.2 μg GAE/g flour. PTS (358.2 μg GAE/g), exhibited the highest total phenolic content followed by GS (291.5 μg GAE/g) and EPS (259.1 μg GAE/g), whereas the minimal quantity of phenolic acids was found in IR64 (79.13 μg GAE/g). Shao et al. (2018) stated that the phenolic content fluctuates from 0.79 to 51 mg GAE/g of rice flour in the pigmented rice genotypes. A significant positive correlation was observed between the phenolic content and other variables such as FRAP ($r = 0.91$), zinc ($r = 0.90$), and potassium ($r = 0.915$) ($p < 0.01$) as shown in Table 2. The correlation results suggested that phenolic content has a substantial role in the secondary metabolites production in rice grains.

Flavonoids are the most abundant classes of secondary metabolites, which exhibit potential antioxidant capacities and have significant health impact on humans (Havsteen, 2002). The flavonoid content differed considerably among the rice genotypes and varied from 70.66 to 151.2 μg rutin eq/g ($p < 0.05$) in the pigmented traditional rice varieties (Table 1). Flavonoid content was found to be maximum in EPS (151.2 mg) followed by PTS (140.7 mg), VS (100.5 mg) and GS (99.54 mg), whereas IR64 (99.54 mg) had the lowest. The differences in the flavonoid contentsmight have been due to the genotypical variations of the respective rice varieties. Mir et al. (2016) stated that substantial differences were observed in the flavonoid contents of the pigmented rice varieties from Kashmir.

3.3. Biological activities of rice varieties

DPPH assay evaluated the free radical scavenging activities of rice extracts and the results are enlisted in Table 3. The inhibitory concentration (IC_{50}) ranged from 1.67 to 8.81 μg/mL and differed considerably among the rice genotypes ($p < 0.05$). The radical scavenging capacity was reported to be maximum for PTS and minimum for IR64. Genetic diversity and climatic variations might also be responsible for differences in the constituents of rice secondary metabolites which have impacts on variation in DPPH radical scavenging activity (Mir et al., 2016; Shao et al., 2018). All the rice varieties displayed a noteworthy correlation between DPPH radical scavenging activity and the total antioxidant capacity as depicted in Table 2.

Reducing power of a biological substance is the capacity to transfer

Table 2
Pearson's correlation coefficients between the minerals and biological activities.

X	Y	Pearson's correlation coefficients, r
FRAP	TPC	0.91[a]
DPPH	TAC	0.90[a]
Zinc	TPC	0.90[a]

[a] Correlation is significant at the 0.05 level (2-tailed).

Table 1
Minerals and total phenols and flavonoids composition of traditional pigmented and commercial rice varieties of Tamil Nadu.

Nutrients	Rice varieties				
	PTS	EPS	VS	GS	IR64
Phosphorus (P)	234.9 ± 0.088^{b}	266.5 ± 0.384^{a}	216.2 ± 0.120^{b}	199.3 ± 0.058c	47.47 ± 0.606d
Potassium (K)	178.4 ± 0.058^{a}	165.3 ± 0.058^{a}	117.8 ± 0.088^{b}	116.2 ± 0.145^{b}	21.65 ± 0.666^{c}
Magnesium (Mg)	92.14 ± 0.012^{a}	97.57 ± 0.062^{a}	76.96 ± 0.063^{b}	61.66 ± 0.091^{c}	22.67 ± 0.636^{d}
Calcium (Ca)	31.93 ± 0.033^{b}	25.76 ± 0.061^{c}	46.04 ± 0.135^{a}	12.2 ± 0.088^{d}	9.11 ± 0.796^{d}
Iron (Fe)	3.097 ± 0.001^{b}	7.395 ± 0.001^{a}	3.539 ± 0.010^{b}	2.479 ± 0.007^{c}	1.310 ± 0.058^{d}
Zinc (Zn)	2.718 ± 0.181^{a}	1.963 ± 0.05^{b}	1.712 ± 0.038^{b}	1.438 ± 0.020^{c}	0.741 ± 0.006^{d}
Manganese (Mn)	0.541 ± 0.001^{c}	1.377 ± 0.011^{a}	0.584 ± 0.002^{c}	0.869 ± 0.002^{b}	0.172 ± 0.015^{d}
Copper (Cu)	0.294 ± 0.001^{b}	0.391 ± 0.001^{a}	0.02 ± 0.001^{d}	0.109 ± 0.001^{c}	0.101 ± 0.006^{c}
TPC	358.2 ± 7.10^{a}	259.1 ± 10.06^{c}	93.2 ± 12.03^{d}	291.5 ± 9.939^{b}	79.13 ± 8.00^{e}
TFC	140.7 ± 4.61^{a}	151.2 ± 8.86^{a}	100.5 ± 7.88^{b}	99.54 ± 6.72^{b}	70.66 ± 5.82^{c}

Values are mean ± standard error of three replicates for each variety (PTS-Puzhuthikar, EPS-Elipaipoo Samba, VS- Valan Samba, GS- Garudan Samba). Different alphabetical letters in each column represent statistically significant differences (Dunnett's test, $p \leq 0.05$). Mineral content represented in mg/100 g of flour DW. TPC represent the total phenolic content in μg GAE/g of dry wt. basis and TFC indicate the total flavonoids content in μg RUE/g of dry wt. basis values.

Table 3
Antioxidant, anti-diabetic assays, and their IC_{50} values for acetone extracts of traditional pigmented and commercial rice varieties.

Biological activities	PTS	EPS	VS	GS	IR64
DPPH Radical scavenging activity	1.667[a]	2.054[a]	3.569[b]	4.52[c]	8.871[d]
FRAP	2.961[a]	2.192[a]	5.189[c]	4.360[b]	12.58[d]
Superoxide anion scavenging activity	3.902[a]	3.426[a]	10.12[c]	9.032[b]	10.32[c]
Total antioxidant activity	3.384[a]	4.12[b]	5.454[c]	7.456[d]	9.261[e]
α-amylase inhibition	2.706[a]	2.008[a]	4.091[b]	5.911[c]	8.420[d]
α-glucosidase inhibition	2.129[a]	2.235[a]	3.917[b]	4.049[c]	10.24[d]

Values are mean ± standard error of three replicates (n = 3). IC_{50} -Inhibitory/effective concentration (μg/mL) was calculated from minimum five different concentrations for each rice variety for each variety (PTS-Puzhuthikar, EPS-Elipaipoo Samba, VS- Valan Samba, GS- Garudan Samba). Different alphabetical letters in each column represent statistically significant differences (Dunnett's test $p \leq 0.05$).

electrons, which may serve as a sign of antioxidant potential. A previous study has described that the antioxidant activity relatively depends on the reducing power of the bioactive compounds (Mir et al., 2016). In this study, there was a substantial difference ($p < 0.05$) among the rice varieties in their reducing potential. The highest reducing power was exhibited by EPS (2.19 μg/mL) which was also associated with the highest total phenolic content and DPPH radical scavenging activity and *vice-versa* in IR64 (12.5 μg/mL) with the lowest reducing power.

The superoxide scavenging activity involves the breakdown of NBT into NBT diformazan via an anion radical, which is an important defense mechanism against superoxide toxicity (Hazra et al., 2008). The superoxide radical scavenging effect (IC_{50}) differed considerably among the varieties ($p < 0.05$) ranging between 3.42 and 10.32 μg/mL (Table 3). The maximum and minimum radical scavenging abilities were observed in EPS and IR64, respectively. The presence of bioactive metabolites in pigmented traditional rice varieties might have been responsible for higher radical scavenging activities as they would play an efficient role in obstructing oxidative damages by superoxide radicals (Valarmathi et al., 2015).

The total antioxidant activity (IC_{50}) ranged from 3.38 to 9.26 μg/mL (Table 3) and differed notably among the varieties ($p < 0.05$). The highest and the lowest radical scavenging capacities were exhibited by PTS and IR64, respectively. Pericarp color and phenolic content are highly correlated with the total antioxidant activity of each rice varieties (Walter et al., 2013). Also, observed a genotypic variations were significantly for antioxidant activities among all the tested traditional rice varieties.

3.4. *In vitro anti-diabetic activities*

Pancreatic α-amylase and intestinal α-glucosidase are key enzymes in carbohydrate metabolism. The inhibitory activity against these enzymes would be essential for lowering the concentration of glucose molecules liberated into the blood circulation system, thereby affecting the glycemic index (GI). This process becomes the base for the proposed mode of action behind the diabetic enzyme inhibitors in reducing GI. These enzymes play a vital part in the hydrolysis of starch in foods and glucose uptake during digestion process (McDougall and Stewart, 2005). In our study, an interesting concentration-dependent relationship was observed between rice extracts and their inhibitory action against α-amylase and α-glucosidase. The inhibitory action of rice extracts (IC_{50}) against anti-diabetic related enzymes α-amylase and α-glucosidase varied from 2 to 8.42 μg/mL and 2.12–10.24 μg/mL, respectively (Table 3). These values differed remarkably ($p < 0.05$) among the traditional pigmented rice varieties. High α-amylase enzyme inhibition was shown by EPS, while inhibition of α-glucosidase was high in PTS. The lowest diabetic enzyme inhibition was exhibited by IR64. Similar to this study, kavuni, a purple rice variety was reported for potential anti-diabetic properties (Valarmathi et al., 2015). Furthermore, phenolics concentration might play a vital role in stimulating inhibitory action against α-amylase and α-glucosidase in the test rice varieties. Consequential reactions such as postprandial glycemic responses, fasting hyperglycemia, improved acute insulin secretion and insulin sensitivity could be alleviated using phenolic rich foods and beverages in diets (Hanhineva et al., 2010; Kim et al., 2010). Pancreatic α-amylase and intestinal α-glucosidase acts on ingested food and further results in starch hydrolysis followed by uptake of glucose. This may lead to rapid increase in glucose level in blood triggering hyperglycemia in type II diabetic patients. Synchronized strong and mild inhibition of α-glucosidase and pancreatic α-amylase might be an efficient approach to manage type II diabetes, respectively (Zhou et al., 2004).

3.5. *Bioactive metabolite profiling of traditional rice varieties*

GC-MS analysis indicated the presence of terpenoids, fatty acids and phytosterols in the pigmented traditional rice extracts (Fig. 1) and their associations are represented as heat map in Fig. 2. The PTS rice extract contained three fatty acids: nonanoic acid, hydnocarpic acid and docosanoic acid. These fatty acids were reported to reveal antibacterial, anti-inflammatory activities, antioxidant, hypocholesterolemic in other plant species (Lima et al., 2005; Geetha et al., 2013; Selvamangai and Bhaskar, 2012). However, EPS rice extract contained two primary bioactive metabolites: Methoxy-nb-alpha-methylcorynantheol along with fatty acids and 4,5-Nonadiene. The presence of these compounds in EPS, might have been responsible for the anti-diabetic, anti-plasmodial activities, anti-cancer and chemo protective properties, respectively (Cao et al., 2011). Previous studies reported that traditional rice varieties would help to improving immunity (Krishnanunni et al., 2015; Valarmathi et al., 2015). Similarly, in this study, EPS which showed higher antioxidant and antidiabetic activities could possibly enhance basal metabolic activities and host immune responses. On contrary, VS rice extracts contained rare terpenoids such as naphthalenone, longipinocarvone, phytol and squalene. Previous studies have reported that phytol (an'monounsaturated acyclic diterpene alcohol) is used a schistosomicide drug, for an endemic tropical disease (de Moraes et al., 2014; Santos et al., 2013). Furthermore, rice extracts of GS exhibited higher fatty acids content. Commonly, fatty acid esters are known to be involved in antioxidant, anti-proliferative, and anti-inflammatory activities (Selvamangai and Bhaskar, 2012). In contrast, IR64 commercial rice variety contained 9 compounds when compared to the traditional varieties. Though, terpenoids, phenols and alkaloids were absent, squalene was detected in smaller quantity. Taking all the parameters together for analysis, four traditional pigmented rice varieties used in this study possessed potential bioactive metabolites which might have influential and notable activities directly or indirectly related to nutritional and medicinal values.

4. Conclusion

The present study has demonstrated that the tested four traditional pigmented rice varieties had an antioxidant potential with the lowest inhibitory concentrations ($p < 0.05$) and the substantial abilities to inhibit diabetic enzymes such as pancreatic α -amylase and intestinal α-glucosidase at 95% confidence interval. There was a significant amount of phenolic content and its association (91%) observed with FRAP activity observed. Subsequently, the metabolite profiling of the traditional pigmented rice varieties showed abundant fatty acids, esters and therapeutically potential metabolites. Although further work is needed to characterize the individual effects of the phytochemical compounds responsible for the antioxidant potentials of the rice extracts, the results of the present study recommend that the traditional pigmented rice varieties can be established as essential nutritive compound sources for the treatment of global health problems such as

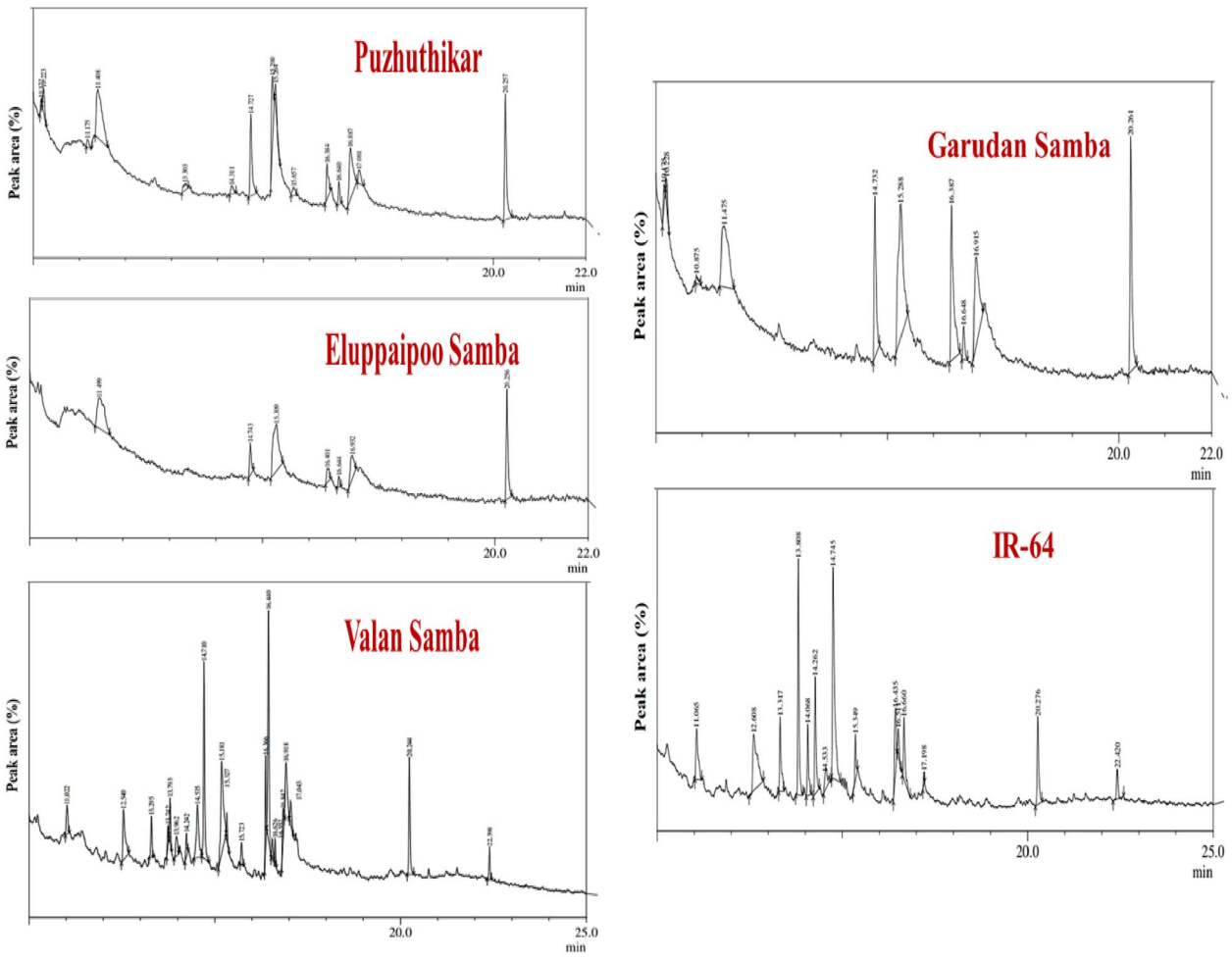

Fig. 1. GC-MS chromatograms of traditional and commercial rice varieties. X axis represents the retention time and Y-axis in GC-MS graph represents the peak area in percentages (%).

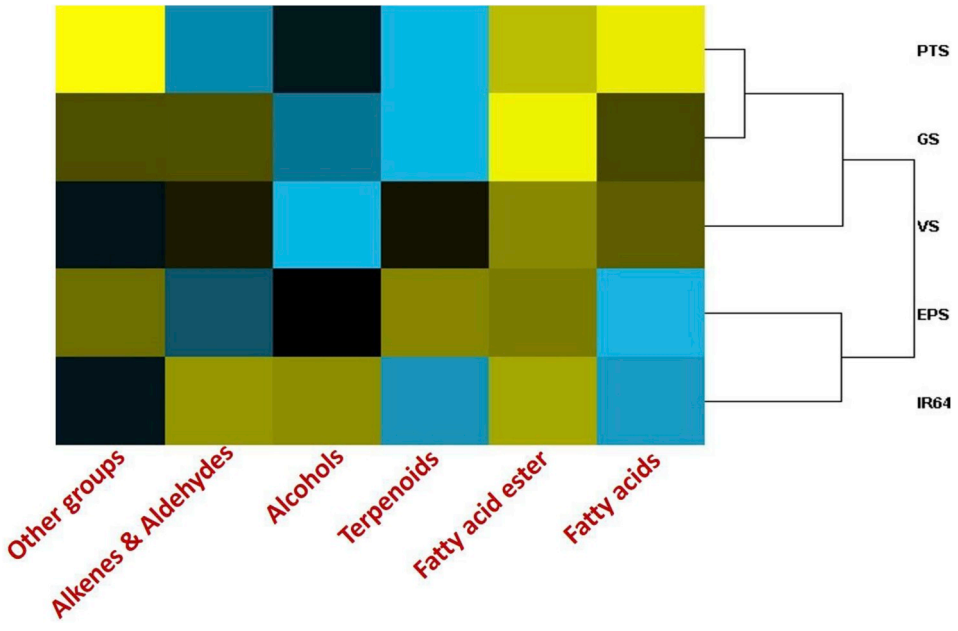

Fig. 2. Heat map association among the metabolites present in traditional and commercial rice varieties.

diabetes and other chronic diseases.

Author's contribution

The work presented here was accomplished with the collaboration of all the authors. The research topic and framework were defined by P. Indra Arulselvi, P. Venkatachalam, T. Senthil Kumar and B. Jayanthi. SR. Manoj and B. Jayanthi prepared the samples for GC-MS analysis under the supervision of T. Senthil Kumar and P. Indra Arulselvi. B. Jayanthi and SR. Manoj prepared the plant material and conducted the biological activity assays. B. Jayanthi analyzed the data and wrote the paper. All the authors revised and approved the manuscript.

Conflicts of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Acknowledgements

All the pigmented rice varieties used in this study were gifted by Mr. S. Senthil Kumar, President Federation for Integrated Improvement in Agriculture Education (FIARE), Attur, Salem. This work was supported by the Department of Science and Technology (DST), India - **Innovation in Science Pursuit for Inspired Research** (INSPIRE) Fellowship [IF120400], Government of India. This work also been sponsored by the University Grants Commission (UGC), India under the **DR D. S. Kothari Postdoctoral Fellowship Scheme (No.F.42/2006 (BSR)/BL/1617/0067).** Authors are thankful to AIRF, Jawaharlal Nehru University (JNU), New Delhi for GC-MS analysis and STIC, Cochin University, Cochin for ICP – AES analysis.

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