

Comparative Hypoglycaemic Index of South Indian Pigmented and Non-pigmented Rice Varieties

G. Chandra Sekar¹, S. Bhagavathy^{2*}

¹Research Scholar, Department of Biomedical Sciences, Faculty of Biomedical Sciences and Technology, Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai – 600 116.

²Assistant Professor, Department of Biomedical Sciences, Faculty of Biomedical Sciences and Technology, Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai – 600 116.

Abstract

Diabetes Mellitus (DM) is the one of the leading chronic metabolic disorders affecting all age groups in universe. Dietary control of diabetes is the main focus of the diabetic patients in order to maintain their blood glucose level. The blood glucose level is mainly affected by carbohydrate and noncarbohydrate foods. Rice is the one of the major foods consumed by most of the Asian countries. There are varieties of rice differ in their chemical nature, nutritional behaviour and phytochemical nature. Based on this, the present work was focused to know the hypoglycaemic effect of pigmented and nonpigmented south Indian rice samples. The quantitative phytochemical analysis was done for Total Polyphenol Content (TPC), Total Flavonoid Content (TFC), and Total Tannin Content (TTC) with the rice samples of Karuppu Kavuni (KK), Mapillai Sampa (MS) and Seeraga Samba (SS), the results show the KK and MS contains high levels polyphenols, flavonoids and tannins than SS. The protein levels were also found in high in KK and MS than SS, in contrast high level of carbohydrate was found in SS than in KK and MS. The GC-MS analysis of the rice extract showed the presence of various bioactive compounds. The antioxidant status and free radical scavenging capacity was also further confirmed KK and MS is good in action than SS. The hypo glycaemic index was done with the control group and type II diabetes patients, shows the KK and MS significantly controls the blood glucose levels and regulates lipoproteins and other biochemical parameters than SS treated groups. The overall results of the present investigation clearly show, that the pigmented rice KK and MS can be considered as good food source in controlling the blood glucose levels and play a vital role in the management and prevention of diabetes and related disorders.

Keywords: Diabetes mellitus, Karuppu Kavuni, Mapillai Samba, Seeraga Samba, Hypoglycaemic effect

1.0 Introduction

Rice (*Oryza sativa*) is a major food consumed by most of the South Asians. Among the rice varieties, pigmented rice received much attention due to their nutrient richness and antioxidant properties [1]. Ali Ghasemzadeh and groups [2] reported the potential impact of antioxidant activity with higher phytochemicals presence in the pigmented rice varieties. Black rice is a types of the species *Oryza sativa* some of which are glutinous rice. The bran hull of black rice contains one of the highest

levels of anthocyanins found in food. The grain has a similar amount of fibre to brown rice and, like brown rice has a mild nutty taste. Black rice has a deep black colour and usually turns deep purple when cooked. Its dark purple colour is primarily due to its anthocyanin content which is higher by weight than that of other colour grains. Black rice is a good source of protein, fibre, calcium, iron, sodium, vitamin C and A, carbohydrate, Magnesium, copper, zinc, potassium, vitamin B₃ and B₂, flavonoids, anthocyanin and other phenolic compounds [3]. Diabetes mellitus is the clinical problem, in which higher blood glucose levels will be observed. This hyperglycaemic condition of diabetes mellitus is the metabolic syndrome, the cells unable to utilize the glucose properly due the lowered Insulin secretion or pancreatic inability to synthesize insulin. This condition gradually leads to increased weight, obesity and elevated blood glucose levels [4]. Glycemic index is the area under the curve of the glucose responses to a carbohydrate-containing food compared to either a specific glucose dose or a specific amount of rice. Food having low glycemic responses has been shown to improve overall blood glucose control in patients with diabetes mellitus and to reduce total serum cholesterol and triglyceride levels in hyperlipidaemia subjects. In Southern India, variety of the pigmented rice were used as a daily intake to reduce the glycemic index especially who ever suffering from diabetes mellitus. Nowadays diet control can be followed by almost all the diseases specifically in diabetes mellitus and obesity to avoid further effects and complications. Food based researchers also provide more information on rise of diabetes with increased intake of rice consumption. This research related with food and health benefits to the human population in general and the people with diabetes in particular. The present study targets to identify the hypo glycemic index of certain rice varieties which can be used as a strategy for diabetic management [5].

2. Materials And Methods

2.1 Collection of *O.sativa*

The fresh seed samples of different varieties of *Oryza sativa* (*O.sativa*) including Karuppu Kavuni (KK), Mappillai Samba (MS), and Seeraga Samba (SS) were randomly collected from nearby organic shop and cleaned with running water dried in sunlight to remove any dust particles and pulverized. The coarse powder stored in air tight container. The seed samples were pulverised into a fine powder and kept in plastic container to avoid any contaminations.

2.2 Extraction of *O.sativa*

The pulverised powder was soaked in solvents - Acetone, Butanol, Chloroform, Diethyl ether, Ethanol, Methanol, and Water (10g of dried powder in 100ml) and extracted for 24h at room temperature with continues shaking. The mixture was centrifuged at 10,000rpm for 15minutes. The supernatant was stored and use for further experiments. Filtrates of the extracts were dried at 40°C. The crude extracts thus obtained was stored in an air-tight container and used for further analysis. This was subjected to the qualitative and quantitative analysis for identification of phyto constituents. 5gm of finely dried and ground seed materials were extracted using 200ml of methanol in Soxhlet apparatus. The extraction was repeated for eight cycles. The extract was filtered through Whatmann No:1 filter paper, concentrated and dried in room temperature. This extract was further used for the GC-MS analysis [6].

2.3 GC-MS Analysis

GC-MS analysis of the methanolic seed extract of *O.sativa* was performed using a Perkin Elmer GC Claurus 500 system and Gas Chromatograph interfaced to a Mass Spectrometer equipped with an Elite 5MS fused silica capillary column (30 × 0.25 mm ID × 1 Mm df, composed of 5% Diphenyl/ 95% Dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 ml/min and an injection volume of 3 µl was employed (split ratio of 10:1). Injector temperature 250°C; Ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min) with an increase of 10°C/min to 200°C, then 5°C/min to 280°C ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV, a scan interval of 0.5 seconds and fragments from 45 to 450Da. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0. Interpretation on mass-spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library and the molecular weight and structure of the components of the test materials were ascertained [7].

2.4 Phytochemical Analysis of *O.sativa*

Quantitative analysis of total phenolic content (TPC) [8], total flavonoid content (TFC) [9], total terpenoid content (TTC) [10], the total protein content and total carbohydrate content were estimated [11] by the standard methods.

2.5 Free Radical Scavenging Activity

Free radical scavenging assay was carried out by DPPH assay [12] and FRAP assay [13].

2.6 Antioxidant Status

The activity of Catalase, [14, 15], Superoxide Dismutase [16, 15], and Glutathione peroxidase [17, 15] were assessed using standard protocols.

2.7 Hypo glycemc Index

2.7.1 Experimental design

The hypo glycemc effect of the selected seeds of *O.sativa* was performed with type II diabetic subjects and control subjects (N=10). The selected subjects were male aged between 30 -50 years with inclusion criteria in a healthy condition, normal body mass index (18.5 - 22.9kg/m²), do not smoke, do not consume alcohol, are not take any medications during the course of experiment. The volunteers were informed consent to express willingness to be the subject of research. The two groups of the subjects were divided into three categories for the consumption of three varieties of rice (A - KK, B - MS and C - SS). All the groups (A, B, and C) consumed reference foods (KK, MS and SS), for the period of 30days. The day before treatment the blood and urine samples were collected. Similarly, 15th day and 30th day blood and urine samples were collected. The collected blood samples were used for the assay of glucose, Cholesterol, triglycerides, lipid profile and HbA_{1C}. Urine sugar was also tested.

2.7.2 Blood Analysis

1. **Estimation of Glucose** : Fasting and postprandial plasma glucose was estimated by the method Kumar and Gill [18].
2. **Determination of HbA_{1C}** : HbA_{1C} analysis was done with Knauer – HPLC Germany (advanced scientific instruments) is a device designed based on affinity chromatography with high function. The needed sample was 4µl of blood, which was centrifuged after addition of the lysing solution. The supernatant was used to be injected into the device. HbA_{1C} measurement was indirectly done based, on the following formula,
$$\text{HbA}_{1C} = 0.58 (\text{Glycosylated Haemoglobin}) + 1.75$$
3. **Estimation of Total Cholesterol** : Total serum cholesterol is estimated by Zak's method. [19].
4. **Estimation of HDL-C** : The blood sample was used for the estimation of HDL-cholesterol by Zak's method [20].
5. **Estimation of LDL-C** : The LDL-C was assayed in the blood sample by the method of David and groups [21].
6. **Estimation of VLDL-C** : The lab can use the triglyceride level to estimate blood VLDL level. The VLDL is about one-fifth of your triglyceride level [22].
$$\text{VLDL-C}(\text{mg/dl}) = \text{Triglycerides}/5$$
7. **Estimation of Triglycerides (TG)** : Estimation of triglycerides was carried out by the method of David R Sullivan and groups [23].

2.7.3 Urine Analysis

Fasting and postprandial urine glucose was estimated by glucose oxidase method. Pipette 0.1ml of urine into 1.8ml of sodium sulphate-zinc sulphate reagent in a centrifuge tube. Add 0.1ml of 2N Sodium hydroxide, centrifuge at 3000rpm for 5 minutes and take 0.5ml of supernatant in duplicate. In the enzymatic determination of urine glucose. The glucose got oxidised to glucose oxidase to gluconic acid and hydrogen peroxide. The enzyme peroxidase converts hydrogen peroxide to water and oxygen. The oxygen in turn reacts with 4-aminophenzone in the presence of phenol to form a red colour complex, intensity of which can be measured at 540nm and compared with that of a standard treated similarly [18].

2.8. Statistical Analysis

The results were expressed as Mean \pm SD and difference among the mean was analysed using One-way ANOVA. The difference in values at $p < 0.05$ was considered statistically significant.

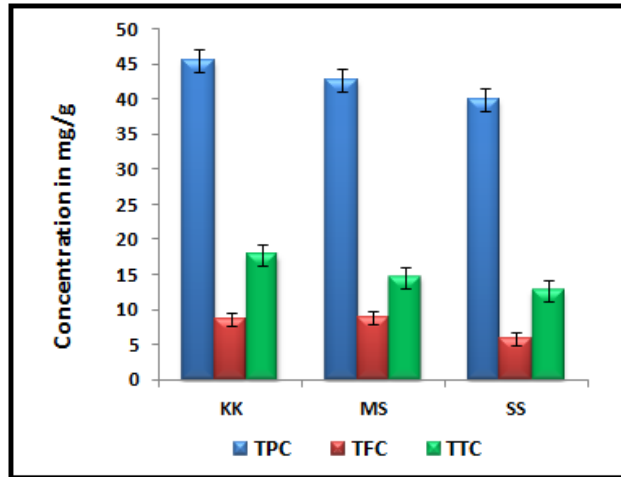
3. Results and Discussion

3.1 Quantitative Phytochemical Analysis

The total phenol, flavonoid and terpenoid contents of *O.sativa* varieties were determined and the quantity was given in **Figure 1**. The total phenolic content and total flavonoid content of methanolic extract of rice varieties – KK, MS and SS were found to be significantly higher compared with standard gallic acid. Phenols and flavonoids of biogenetic origin have proven as excellent antioxidants as well as boost the activity of antioxidant enzymes and protects against oxidative stress. Hence the extract might possess anti-inflammatory, anti-allergic, anti-tumour, antibacterial, anti-fungal, and anti-thrombotic activity. The presence of terpenoids ensures that the extract might boost up the pharmacokinetic properties and also enhance the activity of phenols and flavonoids. Comparatively all the three bioactive

compounds were found to be high in KK and MS than SS, which indicates the consumption of KK and MS may give better regulatory activity than SS.

Figure 1: Quantitative Phytochemical analysis of different varieties of *O. Sativa*

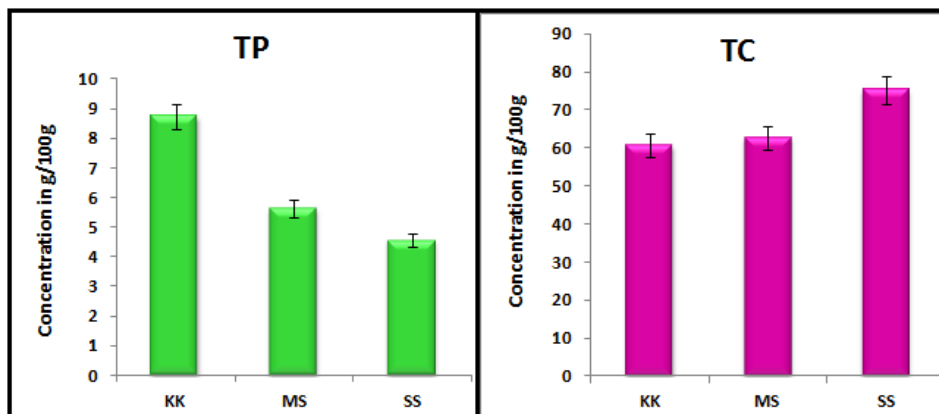


Values are Mean±SD of triplicates

A study on phytonutrients in 10 popular traditional Indian rice landraces stated that total phenolic content of MS has highest total phenol content compared to SS with various rice varieties shown that the brown rice shown high amount of total flavonoid content than white rice [24-26].

Protein amounts detected in raw rice samples were comparable with each other were reported on the **Figure 2**. Normally the *O.sativa* is the good choice for the nutritional carbohydrates than protein, because of their high carbohydrate content. This was also observed in the present result, the total carbohydrate content was found as high in SS rice sample than in KK and MS rice samples. Inversely, while comparing the protein content, the high total protein content was observed in KK and MS than SS. This finding indicates daily consumption of KK and MS may have the nutritional importance in controlling the hyperglycaemic condition and further act as a good protein dietary source.

Figure 2 : Total Protein and Carbohydrate content of different varieties of *O. sativa*



Values are Mean±SD of triplicates

Various previous studies concludes that the white polished rice have higher amount of carbohydrate content than pigmented rice and inversely higher content of protein was identified in brown rice, red rice rather than white rice. A report from different varieties of rice pigmented and non-pigmented rice shown that brown rice has 52g of carbohydrate in 100g, black rice has 34g of carbohydrate in 100g [27], [28].

3.2 GC MS Analysis

The GC-MS analysis of the selected rice varieties was depicted on the **Figure 3** and **Table 1, 2 and 3**.

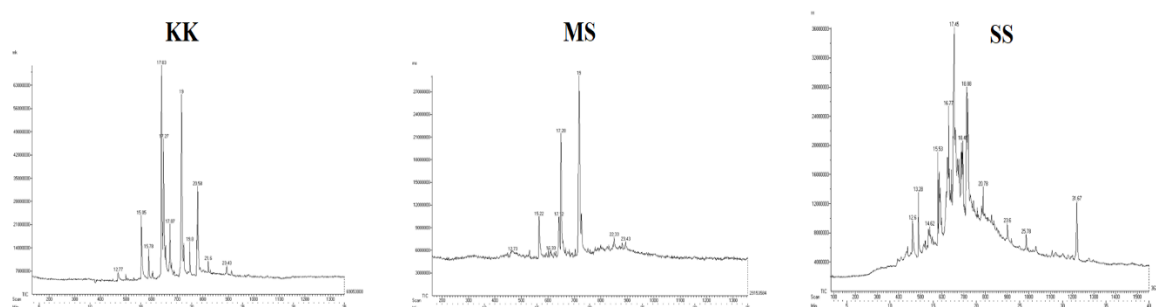



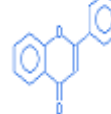

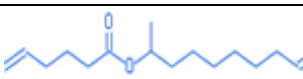







Figure 3 : The GC-MS analysis of KK, MS and SS


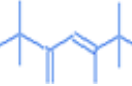
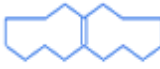
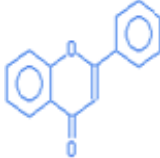

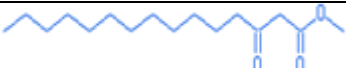


Table 1: GC – MS Analysis of Methanolic extract of KK

S.No.	Compound Name	Retention Time	Molecular Formula	Structure
1.	1-Pentanol	12.77	C ₅ H ₁₂ O	
2.	Nonanal	15.05	C ₉ H ₁₈ O	
3.	Z-2-Tridecen-1-ol	15.78	C ₁₃ H ₂₆ O	
4.	Flavone	17.03	C ₁₅ H ₁₀ O ₂	
5.	Dodecenoic acid, 11-oxo, methyl ester	17.27	C ₁₃ H ₂₄ O ₃	
6.	5-Hexanoic acid (9-decen-2-yl) ester	17.87	C ₁₆ H ₃₂ O ₂	
7.	2-Heptadecanol	19	C ₁₇ H ₃₆ O	
8.	3-Eicosene (E)	19.8	C ₂₀ H ₄₀	
9.	10,12 Octadecadienoic acid, 9 oxo	20.58	C ₁₈ H ₃₀ O ₃	
10.	Diethyl tetradecanedioate	21.6	C ₁₈ H ₃₄ O ₄	
11.	6-methyl-z-6- docosene	23.43	C ₂₃ H ₄₆	

The GC-MS chromatogram of methanol extract of KK shows 11 compounds detected, showed the presence of major peaks and the components corresponding to the peaks were determined as follows. The results revealed that peak 1 – 1-Pentanol at the retention time 12.77, peak 2 – Nonanal at the

retention time 15.05, peak 3 – Z-2-Tridecen – i-ol at the retention time 15.78, peak 4 – Flavone at the retention time 17.03, peak 5 – Do decenoic acid 11 oxo methyl ester at the retention time 17.27, peak 6 – 5 Hexanoic acid at the retention time 17.87, peak 7 – 2 Hepta decanol at the retention time 19, peak 8 – 3 Eicosene the retention time 19.8, peak 9 – 10, 12 Octadecadienoic acid 9 oxo at the retention time 20.58, peak 10 – Diethyl tetra decane dioate at the retention time 21.6, peak 11 – 6 methyl z 6 docosene at the retention time 23.43. The identified compounds nonanal, pentanol and flavones possess biomedical importance possess antimicrobial activity, having antioxidant properties and anti-inflammatory properties. The studies carried out by Zhang and groups [29] confirm the anti-fungal activity of nonanal. 1-pentanol possesses antioxidant role and having pharmaceutical uses. The flavones have anti-inflammatory, anti-allergic, antioxidant, antimicrobial, and anti-osteoporotic activity.

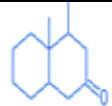


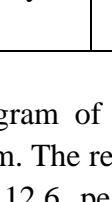
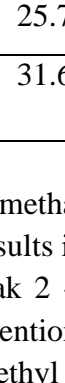
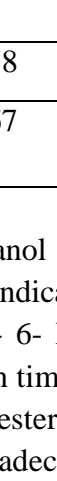
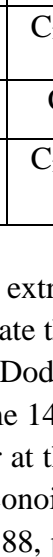
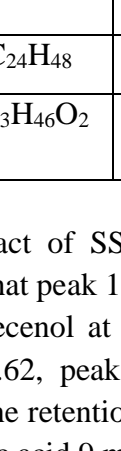
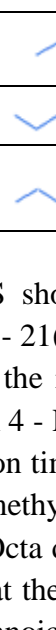

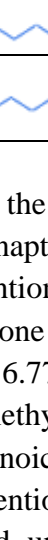
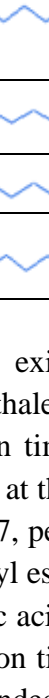
Table 2: GC – MS Analysis of Methanolic extract of MS

No.	Compound Name	Retention Time	Molecular Formula	Structure
1.	Heptane, 2 methyl	12.73	C ₈ H ₁₈	
2.	1,3-pentadiene,2,4 di-t-butyl	13.28	C ₁₃ H ₂₄	
3.	Bicyclo(7.7.0) hexadec-1(9)-ene	16.33	C ₁₆ H ₂₈	
4.	Flavone	17.12	C ₁₅ H ₁₀ O ₂	
5.	n-hexadeconoic acid	17.28	C ₁₆ H ₃₂ O ₂	
6.	Pentadecanoic acid, 3-oxo, methyl ester	19.0	C ₁₆ H ₃₀ O ₃	
7.	Diethyltetradecanedioate	22.33	C ₁₈ H ₃₄ O ₄	
8.	1-Tetradecene, 2 decyl	23.43	C ₂₄ H ₄₈	

The GC-MS chromatogram of methanol extract of MS shows the existence of 8 compounds in the chromatogram. The results indicates that peak 1 – Heptane, 2 methyl at the retention time 12.73, peak 2 – 1,3-pentadiene,2,4 di-t-butyl at the retention time 13.28, peak 3 – Bicyclo(7.7.0) hexadec-1(9)-ene at the retention time 16.33, peak 4 - Flavone at the retention time 17.12 , peak 5 – n -hexadeconoic acid at the retention time 17.28, peak 6 – Pentadecanoic acid, 3-oxo, methyl ester at the retention time 19.0 , peak 7 – Diethyltetradecanedioate at the retention time 22.33, peak 8 – 1-Tetradecene 2 decyl at the retention time 23.43 , peak 8 – at the retention time (**Table 2**). The previous studies supports our present findings which indicates that the presence of the bioactive phytochemicals such as flavones, possess Anti-inflammatory, anti- allergic, antioxidant, antimicrobial and anti-osteoporatic and n-Hexadecanoic

acid act as a good Nematicide, anti-androgenic, antioxidant, pesticide, and 5 alpha reductase inhibitor [30].

Table 3 : GC – MS Analysis of Methanolic extract of SS

S.No.	Compound Name	Retention Time	Molecular Formula	Structure
1.	21(H) naphthalenone. Octahydro-4,4 a-dimethyl	12.6	C ₁₀ H ₁₆ O	
2.	6- Dodecenol	13.28	C ₁₂ H ₂₄ O	
3.	4- Ethylbenzonic acid, allyl ester	14.62	C ₁₂ H ₁₄ O ₂	
4.	Flavone	15.53	C ₁₅ H ₁₀ O ₂	
5.	Dodecanoic acid, 10-methyl; methyl ester	16.77	C ₁₄ H ₂₈ O ₂	
6.	n-Hexadeconoic acid	17.45	C ₁₆ H ₃₂ O ₂	
7.	Heptadeconoic acid,9 methyl, methyl ester	18.45	C ₁₉ H ₃₈ O ₂	
8.	Oleic acid	18.88	C ₁₈ H ₃₂ O ₂	
9.	Octadeconic acid.3 oxo methyl ester	20.78	C ₁₉ H ₃₆ O ₃	
10.	Elaidic acid;isopropyl ester	23.76	C ₂₁ H ₄₀ O ₂	
11.	1-Tetradecene,2decyl	25.78	C ₂₄ H ₄₈	
12.	Dodecanoic acid, undecyl ester	31.67	C ₂₃ H ₄₆ O ₂	

The GC-MS chromatogram of methanol extract of SS shows the existence of 12 different compounds in the chromatogram. The results indicate that peak 1- 21(H) naphthalenone. Octahydro-4,4 a-dimethyl at the retention time 12.6, peak 2 - 6- Dodecenol at the retention time 13.28, peak 3 - 4- Ethylbenzonic acid, allyl ester at the retention time 14.62, peak 4 - Flavone at the retention time 15.53, peak 5 - Dodecanoic acid, 10-methyl; methyl ester at the retention time 16.77, peak 6 - n-Hexadeconoic acid at the retention time 17.45, peak 7 - Heptadeconoic acid,9 methyl, methyl ester at the retention time 18.45, peak 8 - Oleic acid at the retention time 18.88, peak 9 - Octa decanoic acid 3 oxo methyl ester at the retention time 20.78, peak 10 - Elaidic acid;isopropyl ester at the retention time 23.76, peak 11 - 1-Tetradecene,2decyl at the retention time 25.78, peak 12 - Dodecanoic acid, undecyl ester at the retention

time 31.67. The GC-MS results of methanolic extract of SS indicates the presence of bioactive compounds Elaidic acid, isopropyl ester, Dodecanoic acid, undecyl Ester, Flavones, N-Hexadecanoic acid, all these identified compounds are considered as a bioactive compounds through various research findings - anti-inflammatory, hepta preventive, antieczemic, insecticidal activity [31], antimicrobial Activity [32] anti-inflammatory, antiallergic, antioxidant, antimicrobial, anti osteoporotic, Nematicide, antandrogenic, antioxidant, pesticide and 5 Alpha Reductase Inhibitor [7].

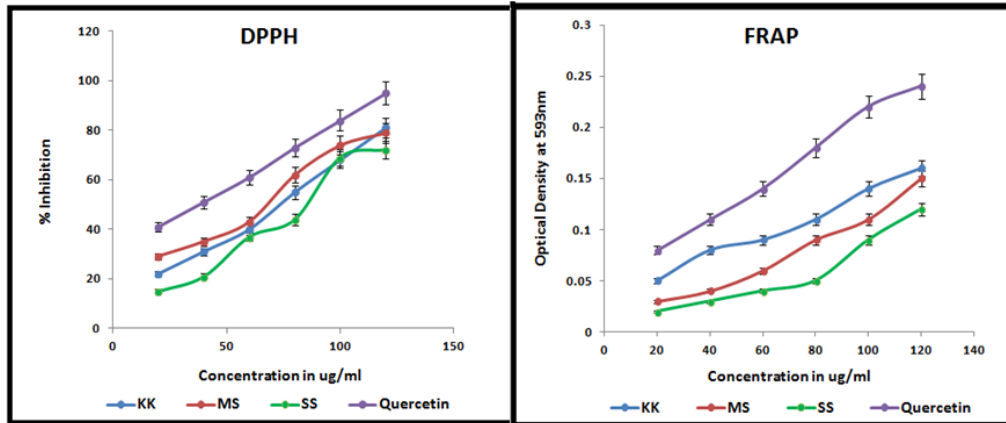
Phytochemicals are plant derived chemicals, beneficial to human health and having the capability of disease prevention. Secondary metabolites from plants are an important source of drugs since ancient times and now almost 50% of the practical drugs used are derived from natural sources. Secondary metabolites of plants like alkaloids, tannins, flavonoids, saponins, anthraquinones, cardiac glycosides and cyanogenic glycosides etc are of pivotal importance.

3.3 Free Radical Scavenging Activity

The result of free radical scavenging activity of the tested rice varieties of *O.sativa* was depicted in the **Figure 4**. The results showed that the bioactive compounds present in the rice extract scavenge more DPPH radicals formed and induce the decolourisation of resultant mixture. The ability of rice extract to reduce more ferric to ferrous ion showed the antioxidant potential of bioactive compounds in the extract. The presence of phytochemicals especially phenols, flavonoids and terpenoids of estimated amount in the *O.sativa* is evident the radical scavenging potential and ensures the ability to fight against disease and might possess varying health benefits. While comparing the results of radical scavenging activities the order of scavenging effects can be indicated as KK > MS > SS. The percentage inhibition was in the order KK-81%, MS-79% and SS-72% of *O.sativa* at a concentration of 120µg/ml. The scavenging effect was increased with increasing concentration. This result provides a direct comparison of the antioxidant activity of quercetin standards. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. Radicals formed in the in the presence of oxygen (ROO·, OH·, RO·, O₂⁻) are highly reactive species greatly differing in their lifetimes and chemical properties. Hence their direct detection is difficult. Stable radical species such as DPPH are often used for the general radical scavenging abilities of antioxidants. The difference in their scavenging capacity may be due to the existence of the bioactive compounds. Hence it is necessary to screen and isolate the biologically active pharmakon for more pharmacological benefits.

Rice extracts of *O.sativa* displayed stronger scavenging efficiency. It can be noticed that the extracts at high concentrations showed significant decrease in the absorption of DPPH radicals when compared with reference standard. From these results this was evident that the ethanol extracts contain bioactive phytochemicals that having hydrogen donating ability and therefore the ethanol extract could serve as free radical scavengers, acting possibly as primary antioxidants. Generally, it is well known that plant phenolics are highly effective free radical scavengers and antioxidants. The reported research findings prove that the phenolics compounds and its derivatives such as phenolic acids and terpenoids were strongly correlated with antioxidants. In the present study also the significant scavenging activity of the extracts may be attributed to the presence of phenolic and flavonoid content. The results indicated that the methanolic extracts of *O.sativa* have significant free radical scavenging activity and thus exhibited the significant antioxidant potential.

Figure 4 : Free radical scavenging activity of different varieties of *O. sativa*



Values are Mean±SD of triplicates

DPPH free radical activity of three Indian medicinal rice varieties are analysed and it resulted that njavara yellow have highest DPPH free radical activity compared to other two rice njavara black and hraswa [34]. Another study based on the extract of different colour rice bran was analysed and it results that black rice bran have highest DPPH activity compared to brown and red rice bran [2]. DPPH activity of brown and white rice was analysed it shown that brown rice have highest DPPH activity compared to white rice [34]. Analysis for black, red and brown colour rice varieties black rice shown the highest and amount of FRAP activity followed by brown and red colour rice. [35].

3.4 Antioxidant Status

The result of antioxidant status of the tested rice samples of *O. sativa* was given in **Figure 5, 6 and 7**. From the results, it is evident that the antioxidant activity was effectively shown by all the three variant rice samples. The tested activity of Catalase, SOD and GPx showed increased activity by increased concentration (20-120µg/ml). Karuppu Kavuni and MS showed almost similar antioxidant activity comparing with SS.

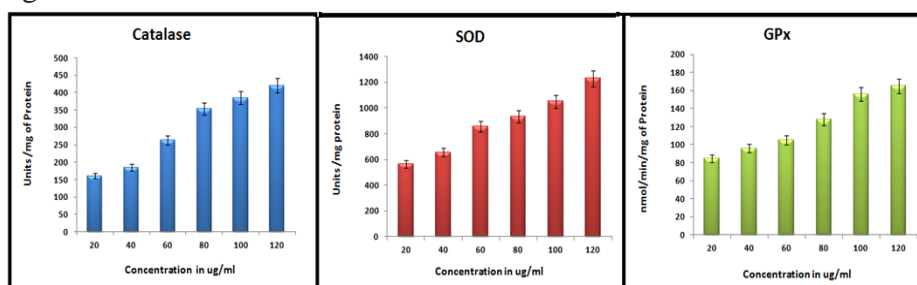


Figure 5 : Antioxidant status of Methanolic Extract of KK

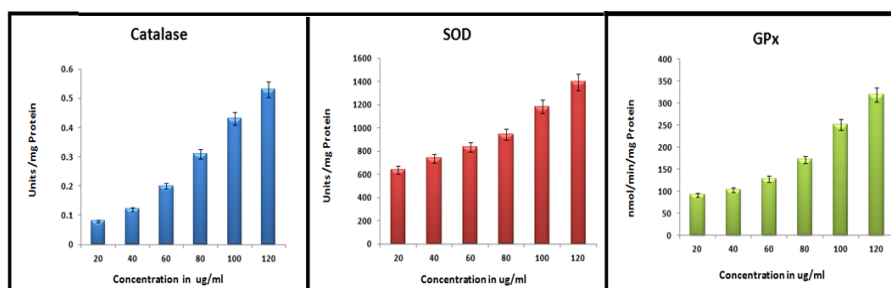


Figure 6 : Antioxidant status of Methanolic Extract of MM

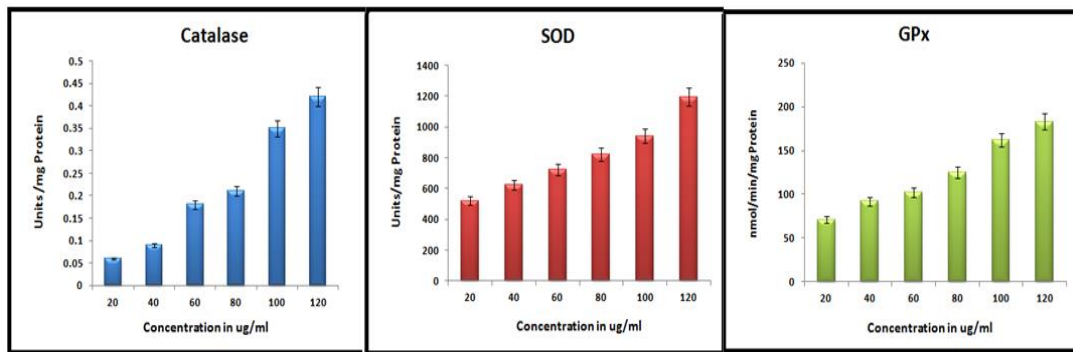


Figure 7 : Antioxidant status of Methanolic Extract of SS

Reports says that the catalytic activity of black rice and white rice extract results that black rice had stronger catalase activity followed by white rice extract. The catalase activity of raw black and white rice, pressure cooked black rice and white rice and conventionally cooked black and white rice was assayed it results that raw black rice have higher value of catalase activity followed by raw white rice. Pressure cooked and conventionally cooked white and black rice have less value of catalase activity compared to raw rice. Superoxide dismutase activity of different varieties of rice was assayed and it results that KHO and MR rice have higher SOD activity and IR64 have lower SOD activity [36]. The glutathione peroxidase activity of raw black and white rice, pressure cooked black rice and white rice and conventionally cooked black and white rice was assayed it results that raw black rice have higher value of GPx activity followed by raw white rice. Pressure cooked and conventionally cooked white and black rice have less value of glutathione peroxidase activity compared to raw rice.

3.5 Hypoglycaemic Index

The results of hypoglycaemic index of the selected rice varieties were depicted in table 4, 5 and 6.

Table 4: Hypoglycemic effect of Karuppu Kavuni

Biochemical Analysis	0 th Day		15 th Day		30 th Day	
	Control	DM	Control	DM	Control	DM
Blood Glucose (F) (mg/dl)	72	177	75	136	71	120
Blood Glucose (PP) (mg/dl)	91	343	89	237	87	179
Mean Blood Glucose (mg/dl)	102	190	91	183	85	173
HbA _{1c} (%)	4.9	8.0	4.8	8.0	4.8	7.8
Triglycerides (mg/dl)	170	131	160	125	157	118
Total Cholesterol (mg/dl)	232	195	218	177	210	171
HDL-C (mg/dl)	45	50	47	51	49	53
LDL-C (mg/dl)	140	120	137	114	135	109
VLDL-C (mg/dl)	35	30	32	25	30	24
Urine Glucose (F)	Nil	Nil	Nil	Nil	Nil	Nil

Urine Glucose (PP)	Nil	++	Nil	+	Nil	+
---------------------------	-----	----	-----	---	-----	---

Control – Healthy individual, **DM** – Diabetes Mellitus (N=10)

Normal Values : **Blood Glucose (F)** -74-99mg/dl; **Blood Glucose (PP)** – Up to 140 mg/dl; **HbA_{1C}** - Normal 4.0-6.0%, Good Control - 6.1-7.0%, Fair Control 7.1-8.0%, Poor Control >8.0; **Triglyceride** – Normal <150mg/dl, Border Line 150-199 mg/dl, High 200-499 mg/dl; **Total Cholesterol** – Desirable <200mg/dl, Border Line 200-250 mg/dl, High > 250mg/dl; Very High >500mg/dl; **HDL-C** - Low <40 mg/dl, High >60 mg/dl; **HDL-** Low <40mg/dl, High >60mg/dl; **LDL** - Optimal <100 mg/dl, Above Optimal 100-129mg/dl, Borderline 130-159mg/dl, High 160-189mg/dl, Very High 190mg/dl; **VLDL** – Up to 30mg/dl; **Urine Glucose (F)** - Nil; **Urine Glucose (PP)** - Nil.

Mapillai Samba (MS) rice had the greater influence on reducing the blood sugar and urine sugar level in the tested diabetic subjects. There was effective decrease in the blood glucose also reduces the mean blood glucose level. The overall percentage of HbA_{1C} also reduced significantly by the regular intake of MS. The lipoprotein profile – triglycerides, total cholesterol, HDL-C, LDL-C and VLDL-C also showed significant variation on 30 days treatment.

Table 5 : Hypoglycemic effect of Mapillai Samba Rice

Biochemical Analysis	0 th Day		15 th Day		30 th Day	
	Control	DM	Control	DM	Control	DM
Blood Glucose (F) (mg/dl)	74	175	76	136	71	122
Blood Glucose (PP) (mg/dl)	98	307	89	246	88	174
Mean Blood Glucose (mg/dl)	110	189	95	171	89	154
HbA_{1C} (%)	4.8	7.7	4.8	7.6	4.8	7.6
Triglycerides (mg/dl)	173	129	162	110	150	104
Total Cholesterol (mg/dl)	235	189	219	177	207	168
HDL-C (mg/dl)	47	50	49	52	50	54
LDL-C (mg/dl)	145	120	136	106	128	104
VLDL-C (mg/dl)	32	27	30	23	29	22
Urine Glucose (F)	Nil	Nil	Nil	Nil	Nil	Nil
Urine Glucose (PP)	Nil	++	Nil	+	Nil	+

Control – Healthy individual, **DM** – Diabetes Mellitus (N=10)

Normal Values : **Blood Glucose (F)** -74-99mg/dl; **Blood Glucose (PP)** – Up to 140 mg/dl; **HbA_{1C}** - Normal 4.0-6.0%, Good Control - 6.1-7.0%, Fair Control 7.1-8.0%, Poor Control >8.0; **Triglyceride** – Normal <150mg/dl, Border Line 150-199 mg/dl, High 200-499 mg/dl; **Total Cholesterol** – Desirable <200mg/dl, Border Line 200-250 mg/dl, High > 250mg/dl; Very High >500mg/dl; **HDL-C** - Low <40 mg/dl, High >60 mg/dl; **HDL-** Low <40mg/dl, High >60mg/dl; **LDL** - Optimal <100 mg/dl, Above Optimal 100-129mg/dl, Borderline 130-159mg/dl, High 160-189mg/dl, Very High 190mg/dl; **VLDL** – Up to 30mg/dl; **Urine Glucose (F)** - Nil; **Urine Glucose (PP)** - Nil.

The SS rice variety shows the less hypoglycaemic effect comparatively KK and MS through the analysis of blood and urine sugar level in the tested diabetic subjects. There was no effectiveness in decreasing the blood glucose was found in the tested samples. This was reflected in the mean blood glucose level. There was fairly controlled level of HbA_{1C} was also identified from the results. There were some in significant variations in the lipoprotein profile - triglycerides, total cholesterol, HDL-C, LDL-C and VLDL-C also detected in the tested DM individuals. This shows the SS rice have no effective influence on its consumption and was unable to reach the effects produced by KK and MS. The diabetic individual the continuous consumption of SS produces the symptoms such as blurry vision, fatigue, being very thirsty and feeling hungry. All these symptoms were normally existed in DM patients, also shows the less hypoglycaemic efficiency of SS. (Table 8)

Table 6 : Hypoglycemic effect of Seerga Samba Rice

Biochemical Analysis	0 th Day		15 th Day		30 th Day	
	Control	DM	Control	DM	Control	DM
Blood Glucose (F) (mg/dl)	74	169	80	190	93	211
Blood Glucose (PP) (mg/dl)	97	316	112	348	129	423
Mean Blood Glucose (mg/dl)	92	160	103	173	112	206
HbA _{1C} (%)	4.8	7.2	4.9	7.4	4.9	7.8
Triglycerides (mg/dl)	176	130	201	152	246	186
Total Cholesterol (mg/dl)	227	193	253	217	289	248
HDL-C (mg/dl)	50	51	47	48	45	44
LDL-C (mg/dl)	136	121	162	147	168	174
VLDL-C (mg/dl)	27	30	31	34	34	40
Urine Glucose (F)	Nil	Nil	Nil	Nil	Nil	Nil
Urine Glucose (PP)	Nil	+	Nil	++	Nil	++

Control – Healthy individual, DM – Diabetes Mellitus (N=10)

Normal Values : Blood Glucose (F) -74-99mg/dl; Blood Glucose (PP) – Up to 140 mg/dl; HbA_{1C} - Normal 4.0-6.0%, Good Control - 6.1-7.0%, Fair Control 7.1-8.0%, Poor Control >8.0; Triglyceride – Normal <150mg/dl, Border Line 150-199 mg/dl, High 200-499 mg/dl; Total Cholesterol – Desirable <200mg/dl, Border Line 200-250 mg/dl, High > 250mg/dl; Very High >500mg/dl; HDL-C - Low <40 mg/dl, High >60 mg/dl; HDL- Low <40mg/dl, High >60mg/dl; LDL - Optimal <100 mg/dl, Above Optimal 100-129mg/dl, Borderline 130-159mg/dl, High 160-189mg/dl, Very High 190mg/dl; VLDL – Up to 30mg/dl; Urine Glucose (F) - Nil; Urine Glucose (PP) - Nil.

The experimental was conducted to analyse the effect of black rice containing anthocyanin on plasma and hepatic parameters in type 2 diabetes mellitus. The results shown that black rice have the hypoglycaemic effect such as total cholesterol level, plasma arteriosclerotic index and hepatic lipids [37]. The open labelled randomized crossover study in and out patients with type 2 diabetes were observed. The results shown that HbA_{1C}, and glycoalbumin decreased significantly when the patients were eating glutinous brown rice, additionally the 30 min postprandial plasma glucose level also

decreased significantly by intake of glutinous brown rice. In contrast there were no changes of glycemic control during the white rice period [38]. Based on the evidence presented in this research, it is clear that dietary components have significant and clinically relevant effects on blood glucose modulation. An integrated approach that includes reducing excess body weight, increased physical activity along with a dietary regime to regulate blood glucose levels will not only be advantages in type 2 DM management, but will benefit the health of the population and limit the increasing worldwide incidence of type 2 DM.

4.0 Conclusion

To conclude, the present work was aimed to know the beneficial effect of different rice varieties of south India in controlling the carbohydrate metabolism and the related other biochemical parameters, in type II diabetic patients and to minimize the effectiveness of the disease based on the food intake. Comparison analysis was done to explore the available biomedical important compounds in the selected rice varieties. The result of the present findings concludes that the rice sample KK and MS are considered as a good choice in regulating the carbohydrate metabolism, possess hypoglycaemic effect and controlling the disease progression in the type II DM. Thus, consuming a diet rich in antioxidant foods will provide health protective effects application in nutritional / pharmaceutical fields, in the prevention of free radical mediated diseases like diabetes mellitus.

Conflict of Interest

The authors do not have any conflict of interest.

Acknowledgement

The authors acknowledge the management of Mohamed Sathak College of Arts and Science, Sholinganallur, Chennai, India. for granting the permission to carry out the research work.

Authors Biography

The authors carried out the present research work during the course of Master of Philosophy project work of the first author in the PG and Research Department of Biochemistry, Mohamed Sathak College of Arts and Science, Sholinganallur, Chennai, India. under the guidance of the corresponding author, the complete research work was carried out by the first author, the data analysis and the paper work was equally shared by both of the authors.

References

1. Ravindra B., Malabadi, Kiran P., Kolkar 1., Raju K., Chalannavar, White and Brown Rice- Nutritional Value And Health Benefits: Arsenic Toxicity In Rice Plants, International Journal of Innovation Scientific Research and Review, 2022, 4 (07), 3065-3082.
2. Ali Ghasemzadeh, Mohamad Taghi Karbalaii, Hawa Z.E., Jaafar and Asmah Rahmat, Phytochemical constituents, antioxidant activity, and antiproliferative properties of black, red, and brown rice bran, Chemistry Central Journal, 2018, 12, (17), 1-13.
3. Nitin Kumar, Roshini Deepika Murali, Black Rice: A Novel Ingredient in Food Processing, Journal of Nutrition and Food Science, 2020, 10 (2), 1-7.
4. Viswanathan Mohan, Donna Spiegelman, Vasudevan Sudha, Rajagopal Gayathri, Biling Hong, Kallingal Praseena, Ranjit Mohan Anjana, Effect of brown rice, white rice, and brown rice with

- legumes on blood glucose and insulin responses in overweight asian Indians: a randomized controlled trial. *Diabetes Technology and Therapeutics*, 2014, 16(5), 317-325.
5. Meera K., Smita M., Sundaramoorthy Haripriya, Soumya Sen, Varietal influence on antioxidant properties and glycemic index of pigmented and non-pigmented rice, *Journal of Cereal Science*, 2019, 87, 202-208.
 6. Adnaik R.S., Pai P.T., Mule S.N., Naikwade N.S., Magdum C.S., Luxative activity of *Vitex negundo* linn leaves, *Asian Journal of Experimental Science*, 2008, 22(1), 159-160.
 7. Yahaya Gavamukulya, GC-MS Analysis of bioactive phytochemicals present in ethanolic extracts of leaves of *Annona muricata*: A further evidence for its medicinal diversity, *Pharmacognosy Journal*, 2015, 7(5), 300-304.
 8. Singleton V.L., Orthofer R., Lamuela-Raventos R.M., Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymology*, 1999, 299, 152-178.
 9. Mervat M.M., El Far, Hanan A., Taie A., Far Mervat M.M. El, Taie Hanan A.A., Antioxidant activities, total anthrocynins, phenolics and flavonoids contents of some sweet potato genotypes under stress of different concentrations of sucrose and sorbitol, *Australian Journal of Basic Applied Science*, 2009, 3, 3609-3616.
 10. Ferguson N.M., A Text book of pharmacognosy. MacMilan Company: New Delhi; 1956., 91.
 11. Rimal Issac, Aparna Nair, Murthy Chavali, and Elize Varghese, Phytochemical, antioxidant and nutrient analysis of medicinal rice varieties found in South India, *Journal of Computational and Theoretical Nanoscience*, *Advanced Science Letters*, 2012, 5, 1-5.
 12. Shimada K., Fujikawa K., Yahara K., Nakamura T., Antioxidative properties of xanthone on the auto oxidation of soybean in cyclodextrin emulsion. *Journal of Agriculture and Food Chemistry*, 1992, 40, 945-948.
 13. Benzie I.F., Choi S.W., Antioxidant in food: content, measyrement, significance, action, cautions, caveats, and research needs, *Advanced Food Nutrients Research*, 2014, 71, 1-53.
 14. Sinha A.K., Colorimetric assay of catalase, *Analytical Biochemistry*, 1972, 47:389-394.
 15. Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J., Protein Measurement with Folin Phenol Reagent. *Journal of Biological Chemistry*, 1951, 193:265-275.
 16. Misra H.P., Fridovich I., The univalent reduction of oxygen by reduced flavins and quinones. *Journal of Biological Chemistry*, 1972, 247:188-192.
 17. Rotruck J.T., Pope A.L., Ganther H.E., Swanson A.B., Hafeman D.G., Hoekstra W.G., Selenium: Biochemical role as a component of glutathione peroxidase. *Science*, 1973, 179:588-590.
 18. Kumar V., Gill K.D., *Basic Concepts in Clinical Biochemistry: A Practical Guide*, Edition, Kindle Edition, 2018.
 19. Charles C Allain, Lucy S Poon, Cicely SG Chan, Richmond W., Paul C.F.U., Enzymatic determination of total serum cholesterol. *Clinical Chemistry*, 1974, 20(4), 470-475.
 20. Warnick G Russell, Alan T. Remaley, Measurement of cholesterol in plasma and other body fluids, *Current Atherosclerosis Reports*, 2001, 3, 404-411.
 21. David A Armbruster, Patricia A Lambert, Estimation of LDL-C: Direct assay of LDL-C Comparison, measurement, and calculation, *Laboratory Medicine*, 1996, 27(9) 613-617.

22. Wilson W.F., Loren A., Zech, Richard E., Gregg, Ernst J., Schaefer, Jeffrey M., Hoeg, Dennis L Sprecher, Bryan Brewer H., Estimation of VLDL cholesterol in hyperlipidemia, *Clinica Chemica Acta*, 1985, 151 (3), 285-291.
23. David R Sullivan, Martin Kohlmeir, Zeger Kruijswijk, Clive E West, Martin B Katan, Determination of serum triglyceride by an accurate enzymatic method not affected by free glycerol, *Clinical Chemistry*, 1985, 31(7),1227-1228.
24. Venkatesh Rajendran, Ishwarya Marichamy, Sathish Sundarajan, amd Sathish Kumar Ramalingam, Phytonutrients analysis in 10 popular traditional Indian rice landraces (*Oryza Sativa L.*), Article in *Journal of Food Measurement and Characterization*, 2018, 12(4), 2598-2606.
25. Battacharya S., Roy S., Qualitative and Quantitative assessment of bioactive phytochemicals in gobindhog and black rice cultivated in West Bengal India, *International Journal of Pharmaceutical Science and Research*, 2018, 9(9), 3845-3851.
26. Roland Bocco, Gandonou Christophe Bernard, Gbaguidi Fernand, Ahouansou Ayide Coffi, Phytochemical screening and quantitative variation of some secondary metabolites in five cultivated rice varieties. *Journal of Applied Biosciences*, 2017, 113, 11146-11157.
27. Rathnapriya. T.S., Ann Raelonine Lincy Eliazer Nelson, Kavitha Ravichandran and Usha Antony, Nutritional and functional properties of coloured rice varieties of South India, a review, *Journal of Ethnic Foods*, 2019, 6, 1-11.
28. Sari, Safitri A., Cairns J.R.K., Fatchiyah F., Virtual screening of black rice anthocyanins as antiobesity through inhibiting TLR4 and JNK pathway DRT, *Journal of Physics – Conference Series*, 2020, 1-8.
29. Zhang J.H., He-Long Sun, Shao-Yang Chen, Li Zeng, Tao-Tao Wang, Anti-fungal activity, Mechanism studies on α -Phellandrene and Nonanal against *Penicillium cyclopium*, 2017, 58(1):13.
30. Yahaya Gavamukulya, Faten Abou-Elella, Fred Wamunyokoli1, Hany A El-Shemy, GC-MS Analysis of Bioactive Phytochemicals Present in Ethanolic Extracts of Leaves of *Annona muricata*, *Pharmacognosy and Natural Products, Pharmacognosy Journal*, 2017, 7 (5), 2015, 300-304.
31. Elaiyaraja A., Chandramohan G., Comparative phytochemical profile of *Indoneesiella echioides* nees leaves using GC-MS, *Journal of Pharmacognosy and Phytochemistry*, 2016, 5(6),158-171.
32. Golamari Siva Reddy, Kamma Srinivasalu, Botla Gunta Mahendran, and Ronda Srinivasa Reddy, Biochemical characterization and anti-microbial activity and purification of glycolipids produced by dodecanoic acid, undecyl ester, *Research Journal of Pharmacy and Technology*, 2018, 11(9), 4066-4073.
33. Reshmi R., Nandhini P.V., Antioxidant activity of Indian medicinal rice Njavara. *International journal of Advanced Engineering, Management and Science*, 2018, 4(3), 141-148.
34. Tiluttama Mudoi, and Priyanka Das, A study on phytochemical, and mineral content of indigenous red rice of Assam, India, *International Journal of Current Microbiological Applied Science*, 2019, 8(4) 1-12.
35. Pengkumsri N., Chaivasut C., Saenjum C., Sirilun S., Sivamaruthi B.S., Sirisattha S., Suwannalert P., Peerajan S., Physiochemical and antioxidative properties of black, brown and red varieties of northern Thailand, *Food Science and Technology*, 2015, 35(2), 331-338.
36. Herath S., Entila F.D., Ella E.S., Baltazar A.M., Johnson A.E., Physiological response and antioxidant activity of rice and grass weed to flooding during germination, *Annals of Sri Lanka Department of Agriculture*, 2017, 19, 321-338.

37. Mitsuru Watanabe, Effect of black rice containing anthocyanin plasma and hepatic parameters in type 2 diabetes mellitus, *Food Science and Technology Research*, 2016, 22(5), 719-725.
38. Nakayama T., Nagai Y., Uehara Y., Nakamura Y., Ishii S., Kato H., Tanaka Y., Eating glutinous brown rice twice a day for 8 weeks improves glycemic control in Japanese patients with diabetes mellitus, *Journal of Nutrition Metabolism*, 2017, 8;7(5):e273.