

Study of chemical composition and volatile compounds along with in-vitro assay of antioxidant activity of two medicinal rice varieties: Karungkuravai and Mappilai samba

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Abstract The objective of this study is to analyze the chemical composition and volatiles present in two medicinal rice varieties of “karungkuravai” and “mappilai samba” and to investigate their total phenolic content and bio activity. Chemical composition of the rice varieties was analyzed using gas chromatography/mass spectroscopy (GC-MS), the volatile compounds are identified using static head space analysis (SHS) followed by GC-MS. Total phenolic content (TPC) is estimated using Folin Ciocalteu colorimetric method, antioxidant assay is done using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay and ferric reducing antioxidant power (FRAP) assay. GC-MS and SHS analysis identified pharmaceutically important phytochemicals present in the rice variety. The compounds like “curlone” and “7-chloro-1,3-dihydro-5-phenyl-1-(trimethylsilyl)-2H-1,4-benzodiazapene-2-one” are identified for the first time in any rice variety. Phenols are found to be present in both rice varieties. Both rice varieties also showed antioxidant activity in both DPPH and FRAP assays and the IC_{50} values were 91.08 $\mu\text{g/ml}$ and 359.93 $\mu\text{g/ml}$ for “karungkuravai” and “mappilai samba”, respectively. The correlation coefficient and regression analysis of total phenolic content with DPPH assay and FRAP assay show significant positive correlation coefficient values and coefficient of regression values.

Keywords Medicinal rice · GC-MS · Static head space · Total phenolic content · Antioxidant assay

Introduction

Rice is the seed of the genus *Oryza* a monocotyledon plant belonging to the grass family *Poaceae*. Rice is a staple crop for more than two-thirds of the world population. Over 2 billion people in Asia derive their 80 % of energy needs from rice, which contains 80 % carbohydrates, 7–8 % protein, 3 % fat and 3 % fiber (Juliano 1985). India is the largest rice growing country accounting for about 2/3rd of world acreage under rice crop. Apart from being a major energy source to over 2 billion people in the world rice is also a medicine (Ahuja et al. 2008; Rahman et al. 2006; Nene 2005). Medicinal value of rice was identified by its early cultivators. In China rice has been used for its healing properties from around 2800 BC, other rice growing areas of the world like Thailand, Myanmar, Malaysia, Indonesia, and India have attributed medicinal properties to rice, and the same have been well documented in their early texts (Ahuja et al. 2008). Indigenous rice varieties are still used in Indian states of Karnataka, Madhya Pradesh, Kerala, Tamil Nadu, Uttar Pradesh, the Western Ghats, and Himachal Pradesh to treat various ailments like skin diseases, blood pressure, fever, paralysis, rheumatism, leucorrhea, as well as used as a health tonic and for increasing lactation (Ahuja et al. 2008). In recent times, unpolished rice has been studied as a rich source of important bioactive compounds and nutraceuticals with numerous potential health functions (Kiing et al. 2009). The major bioactive molecules reported in the rice grain include the phenolic acids, polyphenols, oryzanol, tocopherols, tocotrienols and sterols (Zubair et al. 2012).

Over the past few years, there has been an increasing interest in the study of the antioxidant compounds in grains in relation to health benefit because of their antioxidant activity (Butsat et al. 2009; Lai et al. 2009; Shen et al. 2009; Tananuwong and Tewaruth 2010; Zhang et al. 2010). The rice

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is a rich source of natural antioxidants (Kong and Lee 2010; Xu et al. 2001; Muntana and Prasong 2010; Jang and Xu 2009). Antioxidant activity of pigmented rice and rice bran has been earlier reported (Zhang et al. 2006; Nam et al. 2006; Chung and Shin 2007). There are many indigenous rice varieties that are used for treatment of different ailments. It has been established through studies that many rice varieties have differences in their physiochemical composition (Storck et al. 2005). Limited works have been done on measuring the bioactivity of some of the medicinal rice varieties used in the Indian system of medicine (Rao et al. 2010; Deepa et al. 2008).

The present study was aimed to analyse the phytochemicals and volatiles present in the medicinal rice varieties of “Karungkuravai” and “Mappilai samba” and to further assess their potential bioactivity by estimating total phenolic content and in-vitro antioxidant assays, respectively.

Materials and methods

Rice samples

Paddy samples of “karungkuravai” and “mappilai samba” was procured from organic rice farmers from the district of Tanjavur and Tiruvarur in Tamil Nadu State, India.

GC-MS analysis

Preparation of the extract

Fifty grams of the medicinal rice variety was cleaned and pulverized using pestle and mortar, 2 g of the homogenized powdered rice sample was taken in a centrifuge tube and 20 ml of ethanol (chromatography grade) was added in the tube and mixed for 10 min using cylcomixer. The mixture was then centrifuged at 10,000 rpm for 10 min. The resultant supernatant was filtered using 0.2 micron filter. The filtered supernatant was stored in a glass vial. 1 ml of the filtered supernatant was used for GC-MS analysis. 1 μ l of sample was injected in to the GC column using syringe in split mode.

Instruments and conditions

GC-MS analysis was carried out on Thermo fisher GC-MS/MS Quantum TSQ XLS using DB-5MS (30 m \times 0.25 mm \times 0.25 μ m) 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 80 $^{\circ}$ C, keeping 2 min and then heated to 150 $^{\circ}$ C with 40 $^{\circ}$ C/min, and then to 240 $^{\circ}$ C with 4 $^{\circ}$ C/min, and then to 255 $^{\circ}$ C with 2 $^{\circ}$ C/min, and then 285 $^{\circ}$ C with 4 $^{\circ}$ C/min keeping for 3 min; samples were injected in split injection mode with split ratio of 10. The total

run time was 30 min. Mass spectra were taken at 70 ev and was operated in scan mode from m/z 50–650.

GC-MS analysis

One milliliter of sample in the sample vial was kept in the auto-injector and 1 μ l of sample was injected in to the GC column using syringe in split mode. The injection temperature was kept at 290 $^{\circ}$ C. The sample was injected with split mode using split ratio of 10. Initial column temperature was kept at 80 $^{\circ}$ C for 2 min, and heated to 150 $^{\circ}$ C with 40 $^{\circ}$ C/min then to 240 $^{\circ}$ C with 4 $^{\circ}$ C/min, then to 255 $^{\circ}$ C with 2 $^{\circ}$ C/min and then to 285 $^{\circ}$ C with 4 $^{\circ}$ C/min keeping for 3 min. A 30 m \times 0.25 mm \times 0.25 μ m 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. 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.

Static head space analysis

Sample preparation

The rice sample was cleaned and pulverized using pestle and mortar. 1 g of the pulverized rice sample was taken in head space vial and kept in the incubator for equilibration for 30 min at 105 $^{\circ}$ C. After the extraction period was complete 1 ml of vapour sample was auto injected in to the GC inlet using airtight syringe in split mode.

Instruments and conditions

GC-MS analysis was carried out on Shimadzu QP 2010/MS Quantum TSQ XLS using VF-5MS (30 m \times 0.25 mm \times 0.25 μ m) column, AOC 5000 (make-combipal) with auto injection head space analyser and helium as carrier gas. Carrier gas was used at a flow rate of 1.0 ml/min. Head space incubation temperature was at 105 $^{\circ}$ C, incubation time was for 20 min, syringe temperature at 110 $^{\circ}$ C, injection speed at 150 μ l/sec, injection volume-1 ml vapour through air tight syringe and flush time was 10 s. The initial column temperature in GC-MS was kept at 50 $^{\circ}$ C, and heated to 165 $^{\circ}$ C with 10 $^{\circ}$ C/min, keeping 5 min, and then to 250 $^{\circ}$ C with 15 $^{\circ}$ C/min, keeping 3 min; split injection mode with split ratio of 10 was used. The total run time was 23 min. Interface temperature is 280 $^{\circ}$ C; ion source at 200 $^{\circ}$ C; electron energy is 70 ev; mass scan range (m/z) is 35–350 amu.

SHS- GC- MS analysis

A sample of whole de-husked rice kernels (10 g) was placed in a pestle and mortar and ground to fine powder. One gram of homogenized fine powder was taken in 20 ml head space vial and the vial was sealed with a magnetic crimp cap. The samples were placed in an auto sampler tray, and were maintained at room temperature until analysed. The samples were heated at 105 °C for 20 min in incubator with agitation speed of 250 rpm. After equilibration the vapour sample of volatile compounds of rice was injected in to the gas chromatography column using airtight syringe injector and injection temperature was kept at 110 °C. The sample was injected with Split mode using split ratio of 10. The GC initial column temperature was held at 50 °C, then increased to 165 °C at 10 °C/min, and held for a period of 5 min, then increased to 250 °C at 15 °C/min, and held for a period of 3 min; A 30 m × 0.25 mm × 0.25 µm thickness of VF5-MS 5 % phenyl—95 % di-methyl polysiloxane cross bonded liquid phase capillary column was used with helium (99.995 %) as the carrier gas under a flow rate of 1.0 ml/min in the split mode. The total GC cycle run time consisted of a 25.17 min. The MS was operated in the scan mode from m/z 35 to 350. 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.

Total phenolic content and antioxidant assay

Chemicals

1, 1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma, USA. Folin-Ciocalteu reagent, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), dilute hydrochloric acid, ferric chloride and standards like butylated hydroxyl toluene (BHT), gallic acid and ferrous sulphate was purchased from Hi-Media, Mumbai. Solvents for extraction and others chemicals used were of analytical grade.

Preparation of sample extract

Rice samples were powdered and 50 g of the sample was added to 200 ml solvent (ethanol) in 1:4 ratio (sample: solvent) in a dry conical flask. The flask was then incubated for 48 h in a shaker. After incubation, the extract was collected using Whatman No. 1 filter paper and evaporated below 40 °C. To the residual mixture, solvent was added again and incubated in shaker for 24 h. The extract was collected again using Whatman no. 1 filter paper and evaporated below 40 °C, which was then used for further phytochemical analysis.

Determination of total phenolic content

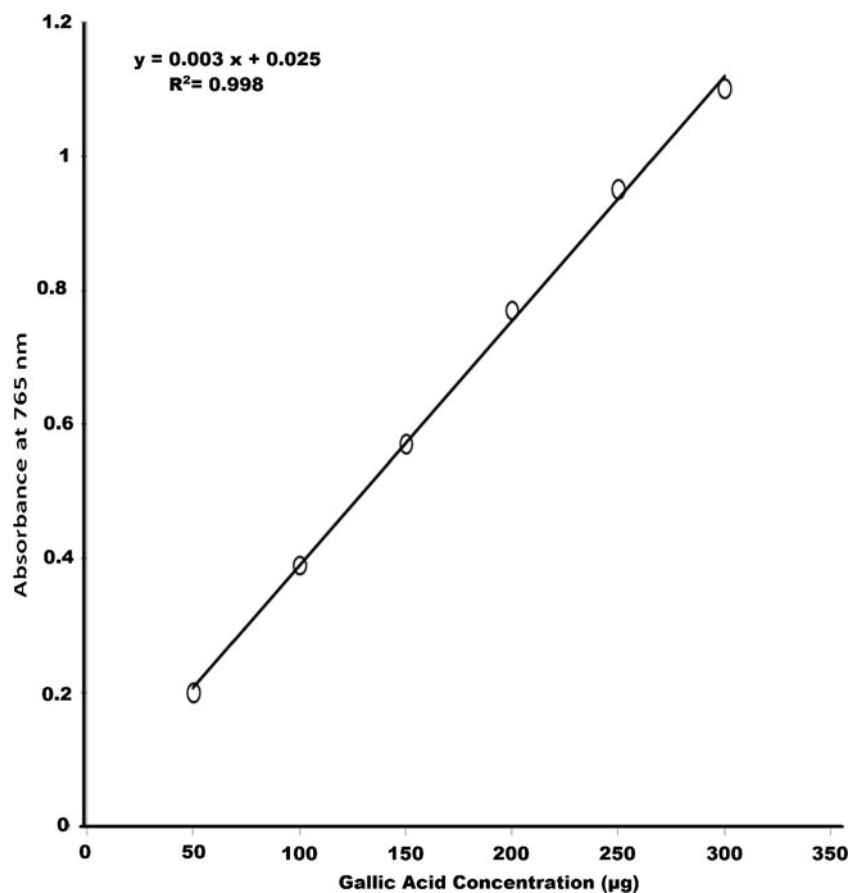
The amount of phenolic compounds in the extracts was determined by the Folin Ciocalteu colorimetric method (McDonald et al. 2001). Calibration curve was prepared using Gallic acid as standard (10 mg/10 ml). From the standard solution 0.05 to 0.2 ml was taken and added to different test tubes. Sample extracts at different concentrations (25, 50, 100, 150 µg) were aliquoted in separate test tubes from the stock solution (1 mg/ml). The volume of the standard and the extract was made up to 1 ml in all the test tubes with distilled water and 5 ml of Folin Ciocalteu (1:10 dilution) reagent was added and the contents were mixed thoroughly. 4 ml of 0.7 M sodium carbonate was added to the mixture after 2 min and was incubated for 30 min. The absorbance was measured at 765 nm in a UV-visible spectrophotometer. The amount of phenolic compounds in the extracts was determined by extrapolating the absorbance of the sample extract on the calibration curve (Fig. 1) obtained with Gallic acid as standard.

Antioxidant assay

FRAP assay Antioxidant activity was measured by ferric reducing antioxidant power (FRAP) assay of Benzie and Strain (1996). FRAP assays uses antioxidants as reductant in a redox-linked colorimetric method, employing an easily reduced oxidant system present in stoichiometric excess. At low pH, reduction of ferric tripyridyl triazine (Fe III TPTZ) complex to ferrous form (which has intense blue color) can be monitored by measuring the change in absorption at 593 nm. The change in absorbance was directly linked to the combined or total reducing power of the electron donating antioxidants present in the reaction mixture. The FRAP reagent was prepared by mixing acetate buffer (300 mM, pH 3.6) with TPTZ (2, 4, 6-tri (2-pyridyl)-s-triazine) (10 mM in 40 mM dilute HCl) and ferric chloride (20 mM) in the ratio of 10:1:1. Ferrous sulphate (1 mM) was used as standard. Various concentrations (25, 50, 100, 150 µg) of the sample was aliquoted from the sample extract stock (1 mg/ml) and made up to 1 ml with distilled water and was mixed with 1.5 ml of working FRAP reagent and incubated at 37 °C for 4 min. After incubation the absorbance was measured at 593 nm. Ferrous sulphate standard was processed in the same way and calibration curve (Fig. 2) was generated using various concentrations of ferrous sulphate (100–500 µg). The FRAP value of the extract was calculated from the standard graph.

DPPH assay The antioxidant activity of the plant extracts and the standard was assessed on the basis of the radical scavenging effect of the Stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH)-free radical according to Clemente and Desai

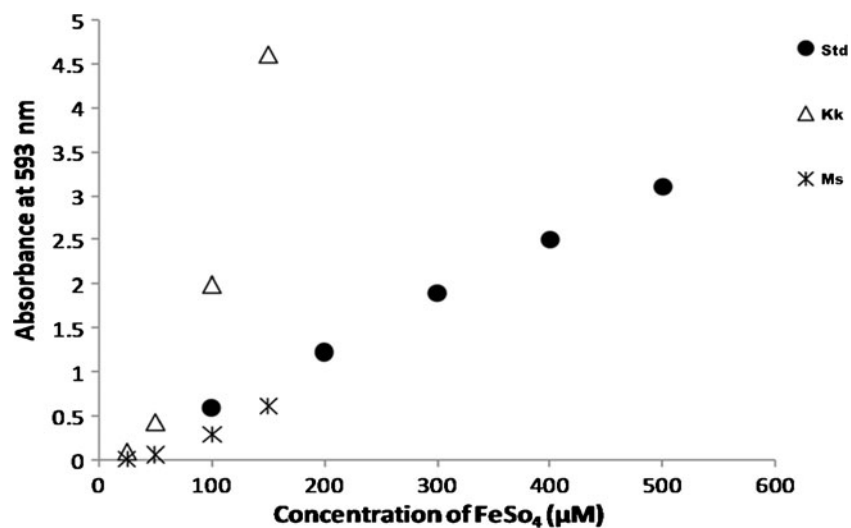
Fig. 1 Gallic acid equivalents in total phenol assay



(2012). DPPH is a stable free radical with purple color (absorbed at 517 nm). If free radicals have been scavenged, DPPH will degenerate to yellow color. This assay uses this character to show the free radical scavenging activity. The sample and standard (BHT 0.16 %) were dissolved in methanol (1 mg/ml). From this stock solution various

concentrations (25, 50, 100, 150, 200 & 250 µg) was aliquoted which was then used to determine the antioxidant potential. 0.1 % of DPPH was prepared in methanol and 100 µl of this solution was mixed with 2.9 ml of sample solution and standard solution at different concentrations separately. These solution mixtures were kept in dark for

Fig. 2 FRAP assay for rice varieties (*Std* standard; *Kk* Karungkuravai; *Ms* Mappilai samba)



30 min and optical density was measured at 517 nm. Methanol with DPPH solution (0.1 %) was used as control. The

optical density was recorded and % inhibition was calculated using the formula given below:

$$\text{Inhibition \%} = \frac{[\text{Absorbance}(A_{517 \text{ nm}}) \text{ of control} \times \text{Absorbance}(A_{517 \text{ nm}}) \text{ of sample}] \times 100}{\text{Absorbance}(A_{517 \text{ nm}}) \text{ of control}}$$

IC₅₀ values (concentration of extract required to scavenge 50 % of free radicals) were calculated by log probit analysis (Kumar and Gowda 2011).

Statistical analysis

All the tests were performed in triplicate ($n=3$) and values were expressed as mean \pm standard deviation. The data were statistically analysed using analysis of variance (ANOVA). The statistical differences between means were analyzed using ANOVA followed by Tukey's least significant difference (LSD) test. The Pearson's correlation analysis and regression analysis was done using StatPlus v2009. IC₅₀ was calculated by log probit analysis using StatPlus v2009. $P<0.05$ was considered statistically significant.

Results & discussion

The rice varieties of "karungkuravai" and "mappilai samba" are traditional rice varieties of Tamil Nadu in southern India. "Karungkuravai" has been traditionally used for curing filariasis and "mappilai samba" is used for imparting strength and for their neurological properties (Arumugasamy et al. 2001, 2002).

Gas chromatography

A total of nine compounds were identified in both rice varieties using GC-MS.

Karungkuravai

The GC-MS chromatogram of "karungkuravai" rice variety showed the presence of eight phytochemicals that are identified using NIST library. Each peak as shown in the chromatogram identifies a compound present in the rice variety. Table 1 lists the phytochemicals identified in rice variety and the biomedical activity associated with them. Chromatogram of this rice variety is shown in Fig. 3a. The phytochemical profile indicates the presence of medicinal activity in the rice variety.

Mappilai Samba



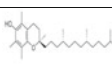
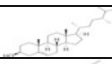
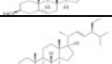
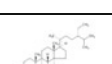
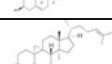
The chromatogram of "Mappilai Samba" rice variety showed the presence of seven phytochemicals these phytochemical compounds were identified using NIST library. Table 1 lists the phytochemicals identified in rice variety and the biomedical activity associated with them. Chromatogram of this rice variety is shown in Fig. 3b. The phytochemical compounds identified signify the medicinal activity of this rice variety.

Out of the phytochemicals identified in the rice varieties the majority belong to the class of chemicals called phytosterols (plants sterols). Out of the phytosterols identified Sitosterol, Stigmasterol and Campesterol are present in both rice varieties. The peak area percentage of Sitosterol is seen to be high in both the rice varieties. Cycloartenol was seen to be present in "Mappilai Samba" and not in "Karungkuravai" rice. Among different plant sterols, sitosterol has been most intensively investigated and has been shown to exhibit anti-inflammatory; antineoplastic, antipyretic, and immunomodulating activities (Careri et al. 2006). Also, sitosterol, campesterol and stigmasterol exert antioxidant effects (Cherif 2012). Squalene, an isoprenoid compound structurally similar to beta-carotene, is an intermediate metabolite in the synthesis of cholesterol. It has been primarily sourced from shark liver oils. Due to concerns for protection of marine life forms, there has been growing interest in extraction of squalene from plant sources. Squalene appears to function in the skin as a quencher of singlet oxygen, protecting human skin surface from lipid peroxidation due to exposure to UV and other sources of ionizing radiation (Kohno et al. 1995). Among the other phytochemicals noted in rice, the ester of stearic acid is present in the rice variety of "mappilai samba" than in "karungkuravai". Tocopherol was noted to be present only in "karungkuravai" and not in "mappilai samba".

Static head space analysis

Volatile profile of both rice varieties were identified using the head space analysis. Table 2 lists the volatile compounds identified. A total of 36 volatile compounds were identified in both rice varieties. Total ion chromatogram of SHS GC of both rice varieties is shown in Fig. 4. Twenty-four volatile compounds were identified in "karungkuravai" rice variety

Table 1 Phytochemicals identified in ethanolic extract of medicinal rice “Karungkuravai” and “Mappilai Samba” and their known medical bioactivity

Sl. No	Retention Time	Similarity Index	Retention Index	Molecular Weight	Name of the compound	Area (%)		Structure	Known Medical Bioactivity
						Karungkuravai	Mappilai Samba		
1	11.665	84	1444	151	2-(hydroxymethyl)-2-nitro-1,3-Propanediol	41.50±0.97			
2	20.193	77	2366	340	Glycidol stearate	2.87±0.23 ^a	6.26±0.37 ^b		5-Alpha-Reductase-Inhibitor, Hypocholesterolemic
3	21.665	85		266	9-Octadecanal,(Z)-	20.11±0.21 ^a 42.61±0.60 ^b			
4	24.111	89	2914	410	Squalene	1.55±0.08 ^a	3.50±0.30 ^b		Antibacterial, Antioxidant, Cancer-Preventive; Immunostimulant, Lipoxygenase-Inhibitor;
5	26.341	86	3149	430	dl-.alpha.-Tocopherol	1.36±0.06			Antialzheimeran, Antianginal, Antiarthritic; Antiatherosclerotic, Anticancer, Anticonvulsant, Antidementia,Antidiabetic, Antiinfertility,Antiischemic, Antimutagenic,Antineuropathic, Antioxidant, Antiparkinsonian,Cardioprotective.
6	27.232	81	2632	400	Campesterol	6.86±0.305 ^a	10.1±0.12 ^b		Antioxidant Hypocholesterolemic
7	27.443	80	2739	412	Stigmasterol	6.53±0.28 ^a	7.10±0.14 ^b		Antihepatotoxic, Antiinflammatory, Antinociceptive Antiophidic,Antioxidant, Antiviral,Artemicide Cancer-Preventive,Estrogenic, Hypocholesterolemic Ovulant
8	27.983	87	2731	414	Gamma-Sitosterol	19.34±0.61 ^a	22.71±0.73 ^b		Hypocholesterolemic, Cancer preventive*
9	28.803	85	2816	426	Cycloartenol		7.59±0.38		Antibacterial , Anti-inflammatory, Antirheumatic Hypocholesterolemic

Data is mean ± standard deviation; Common volatiles were statistically analysed using ANOVA and Tukey's test. Means in the same rows with different superscripts are significantly different, ($p < 0.05$). ANOVA- Analysis of variance

(Fig. 4a) and 25 volatile compounds were identified in “mappilai samba” rice variety (Fig. 4b). Compounds identified are mainly volatile flavours already reported to be present in rice (Duke 1992). In the “karungkuravai” rice variety a volatile compound curlone (21.88 %, mass spectra and structure shown in Fig. 5a) has been identified. Curlone is a sesquiterpene found as a major constituent in turmeric and essential oils of turmeric. The peak area percentage of curlone present in this rice variety is similar to the peak area percentage of curlone found in turmeric (Jayaprakasha et al. 2002; Singh et al. 2010). Curlone is known to exert bioactivity like antioxidant activity, antitumor activity etc. (Jayaprakasha et al. 2002; Kiso et al. 1983). This is the first time curlone is reported to be present in any rice variety. Volatile phytochemical 2H-1,4-Benzodiazepin-2-one, 7-chloro-1,3-dihydro-5-phenyl-1-(trimethylsilyl)- (13.40 %, mass spectra and structure shown in Fig. 5b) has been identified in the rice variety of “Mappilai samba”. This compound has been shown to be present in rice as a siloxane derivative

contaminant when extracted using solid phase micro extraction (SPME) method (Bryant and McClung 2011), however in this case the extraction has been done using static head space method and not by using solid phase micro extraction fibers and therefore the compound cannot possibly be a contaminant from SPME fiber. The rice variety of “Mappilai Samba” is used by some naturotherapist for curing of diseases related to pathology of central nervous system. The presence of the core molecule 1, 4-benzodiazepine-2-one which is a known anxiolytic, anticonvulsant, sedative, hypnotic, and muscle relaxant (Imanzadeh et al. 2011; Drummer 2002) in this rice may be the reason for the neurological beneficial effect noted in this rice variety.

Total phenolic content and antioxidant assay

Bioactivity of the rice varieties were assessed by analysing for the presence of total phenolic content and antioxidant activity.

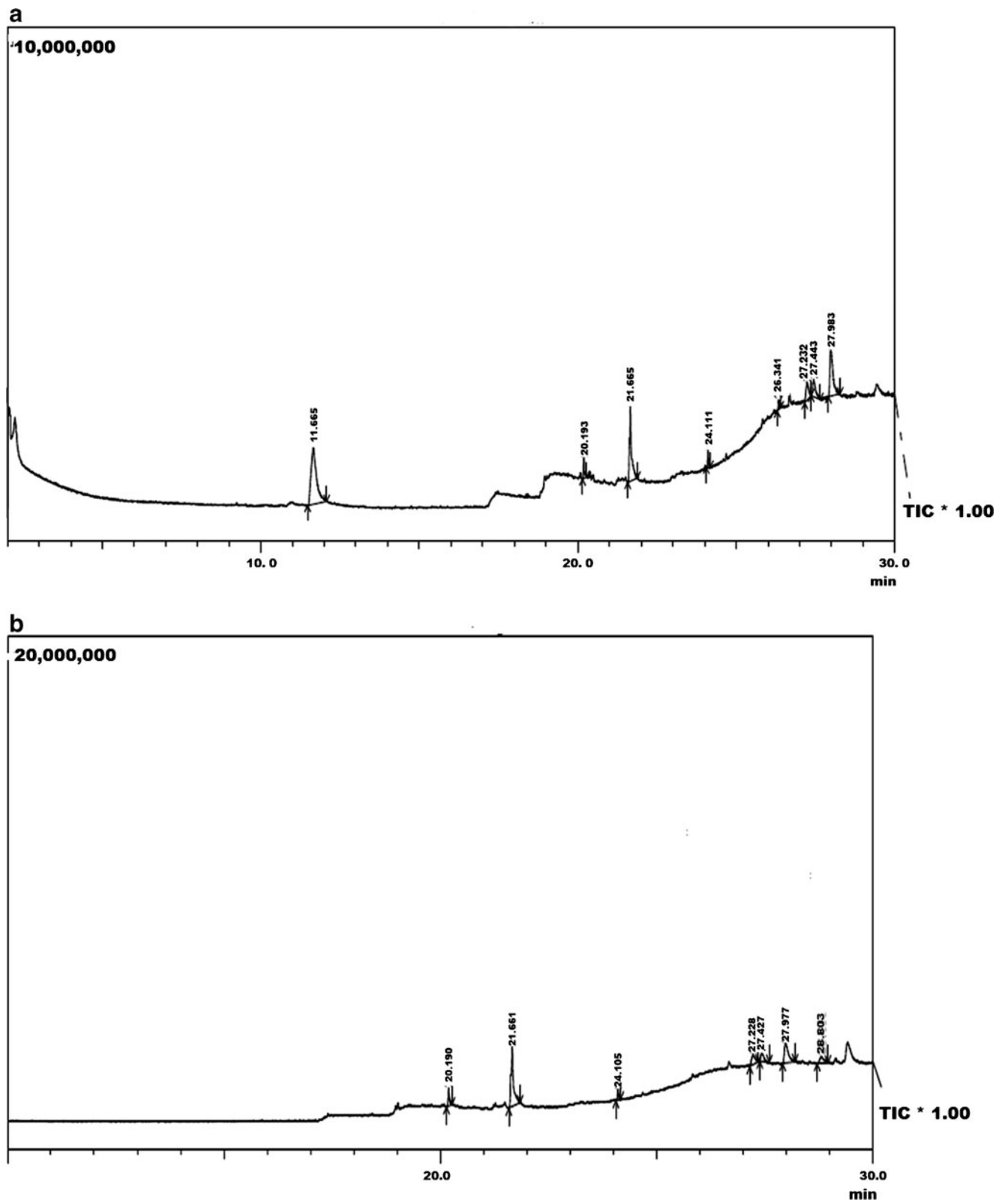


Fig. 3 GC-MS analysis of a Karungkuravai and b Mappilai samba rice varieties

Table 2 Comparative table of various volatile compounds identified using HS-GC-MS in Medicinal Rice “Karungkuravi” and “Mappilai Samba”

Sl.No.	Retention time	Similarity index	Retention index	Name of the compound	Area (%)	
					Karungkuravai	Mappilai Samba
1	1.222	93	1097	l-alanine ethylamide	1.54±0.42	–
2	1.626	99	408	Acetaldehyde	5.37±0.46 ^a	0.42±0.04 ^b
3	2.233	99	463	Ethanol	0.82±0.03 ^a	0.05±0.01 ^b
4	2.62	98	455	Acetone	3.55±0.31 ^a	0.46±0.06 ^b
5	2.719	95	482	2-propanol	0.61±0.08	–
6	2.985	99	465	Cyanomethane	8.37±0.45 ^a	0.83±0.16 ^b
7	3.619	96	543	Isobutyraldehyde	4.13±0.70 ^a	0.28±0.03 ^b
8	4.921	96	586	Ethyl acetate	0.95±0.07 ^a	0.47±0.11 ^b
9	6.533	97	643	Beta-methyl butanal (Isovaleral)	2.79±0.37 ^a	0.18±0.03 ^b
10	6.76	95	643	Alpha-methyl butanal	1.94±0.05 ^a	0.13±0.04 ^b
11	8.127	97	681	2-pentanol	1.6±0.23 ^a	0.07±0.01 ^b
12	10.542	96	806	Hexanal	2.37±0.37 ^a	0.15±0.03 ^b
13	14.757	90	1018	D-limonene	1.85±0.14	–
14	14.888	93	1086	3,7-dimethyl decane	11.48±0.61 ^a	0.14±0.03 ^b
15	14.998	93	1185	3,7-dimethyl-undecane	3.86±0.46	–
16	15.601	90	958	2,3,6,7-tetramethyloctane	1.60±0.08 ^a	0.13±0.04 ^b
17	16.601	91	1104	Nonanal	0.65±0.08	–
18	18.005	95	1582	Curlone	21.88±0.94	–
19	18.618	91	1285	4,6-dimethyldodecane	2.61±0.42	–
20	21.771	83	1494	4,11,11-trimethyl-8-methylene bicyclo(7.2.0) undec-4-ene	3.88±0.27	–
21	22.24	92	1454	5-(1,5-dimethyl-4-hexenyl)-2-methyl-[S-(R*,S*)]-1,3-cyclohexadiene	6.93±0.39 ^a	0.11±0.05 ^b
22	22.358	87	1500	1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (S)-cyclohexene	2.34±0.44	–
23	22.589	93	1446	3-(1,5-dimethyl-4-hexenyl)-6-methylene-[S-(R*,S*)]-cyclohexene	6.26±0.38	–
24	23.108	89	1564	.+/.-trans-nerolidol	2.98±0.12	–
25	3.147	97	511	2-methyl-2-propanol	–	0.22±0.02
26	4.759	98	369	Trimethyl-silanol	–	1.88±0.23
27	13.22	89		2,2-dimethyl-3-methylene-(Delta 2-carene)- bicyclo[2.2.1]heptane	–	0.03±0.01
28	13.442	98	827	Octamethyl-cyclotetrasiloxane	–	42.53±1.05
29	13.746	95	1130	2,2-dimethyl-decane	–	0.1±0.04
30	14.236	85	620	Hexamethyl cyclotrisiloxane	–	0.65±0.09
31	16.232	80	2274	N-[(pentafluorophenyl)methylene]-.beta.,4-bis[(trimethylsilyl)oxy]-benzeneethanamine.	–	37.3±1.22
32	19.091	71	2579	7-chloro-1,3-dihydro-5-phenyl-1-(trimethylsilyl)-2H-1,4-benzodiazepin-2-one	–	13.40±0.44
33	19.48	89	732	3,3-dimethyl hexane	–	0.02±0.005
34	19.68	75	1230	4-(1-methylethyl)-benzaldehyde	–	0.08±0.02
35	21.41	94	1367	1,7,7-trimethylbicyclo[2.2.1]hept-2-yl ester, exo-2-propenoic acid	–	0.28±0.03
36	22.58	85	1398	Cedrene	–	0.15±0.02

Data's were statistically analysed using ANOVA and Tukey's test. Means in the same columns with different superscripts are significantly different, ($p < 0.05$)

Total phenolic content (TPC)

The total phenolic content (TPC), for 100 µg of rice sample extract, expressed as Gallic acid equivalents are shown in

Table 3. Several phenol compounds have already been identified in rice (Walter and Marchesan 2011). The rice variety of “Karungkuravai” exhibited a good quantity of phenolic content when compared to “Mappilai Samba”. For all the

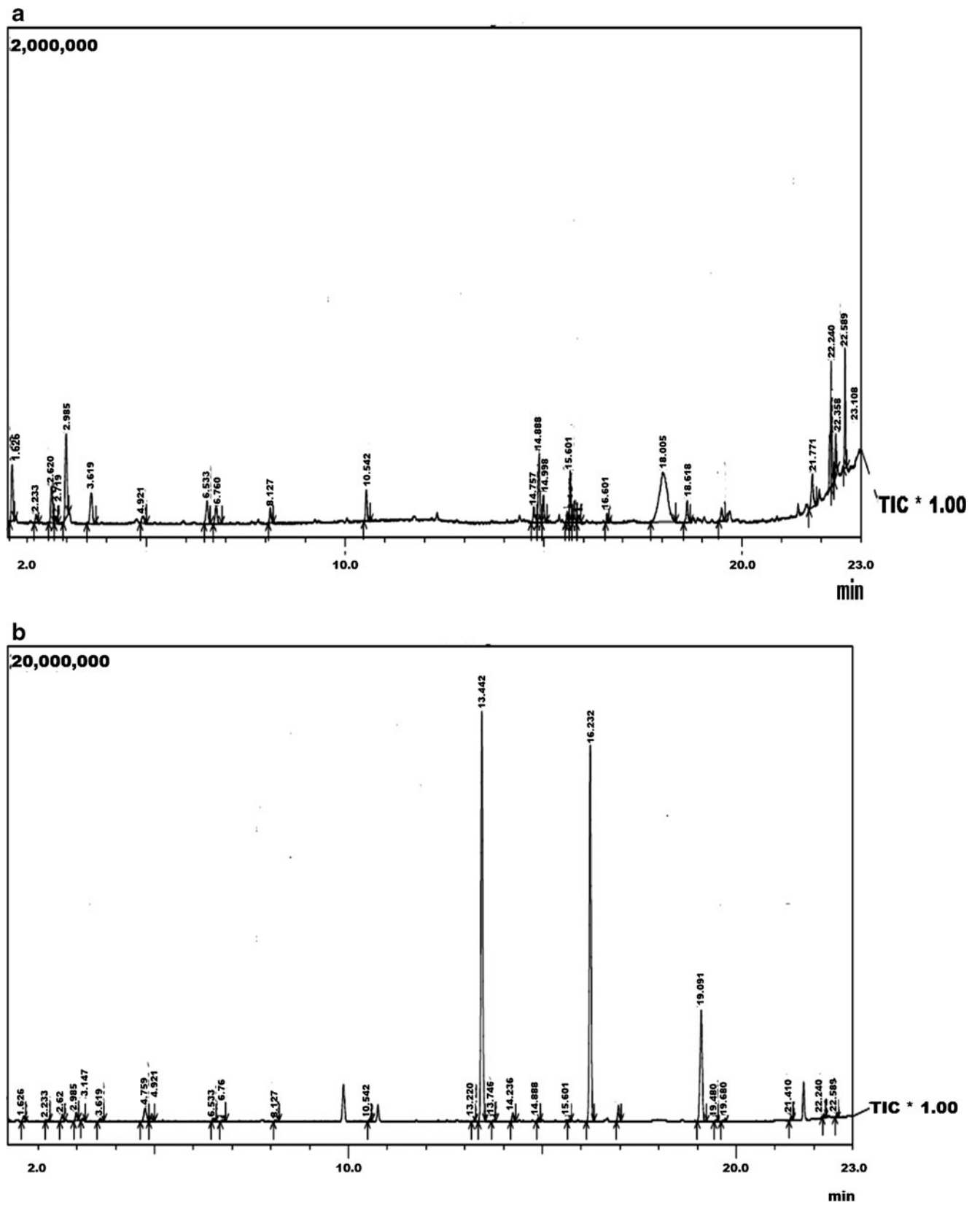
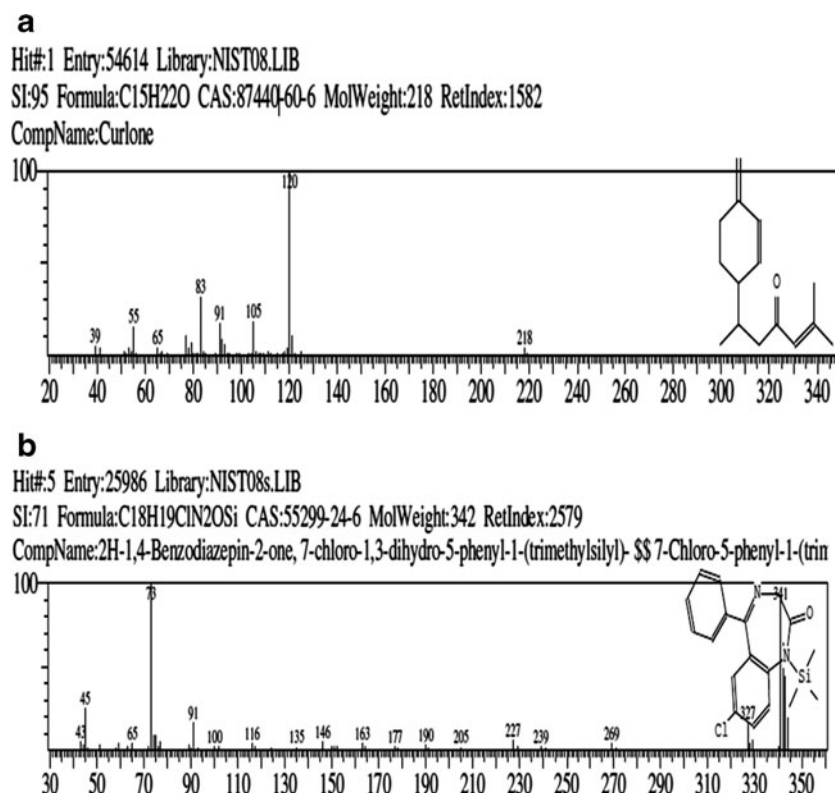


Fig. 4 Static head space analysis of **a** Karungkuravai and **b** Mappilai samba rice varieties

Fig. 5 Mass spectra analysis of **a** Karungkuravai and **b** Mappilai samba rice varieties



concentrations of sample extract, the total phenolic content of “karungkuravai” ($80.25 \pm 1.39 \mu\text{g GAE/g}$) showed highest phenolic content, this rice variety also exhibited a high FRAP value when compared to other rice variety. Though the rice variety of “Mappilai samba” showed presence of phenolic content, it recorded the lowest FRAP value and a high IC_{50} value. In both the rice varieties increase in concentration also showed an increase in the phenolic content value.

Antioxidant assay

FRAP assay The ability of the rice extract to reduce the ferric ions was determined using FRAP assay. Figure 2 shows the optical density (O.D.) values of rice varieties. The “Karungkuravai” rice variety had higher OD value than that of the standard (FeSO_4) at $100 \mu\text{M}$ concentration, while the O.D. value of “Mappilai samba” was lower than that of the

standard at $100 \mu\text{M}$ concentration. FRAP value was noted to be high in the rice variety of “Karungkuravai” ($3181 \pm 16.52 \mu\text{M/mg}$). FRAP value (at $100 \mu\text{g}$ of rice sample extract) of rice varieties are shown in Table 3.

DPPH assay Free radical scavenging activities of the rice sample extracts were assessed by the DPPH assay. Figure 6 showing the standard graph plotting the various concentrations of rice extracts against the percentage inhibition, demonstrates a significant decrease in the concentration of DPPH radical due to scavenging ability of the rice extracts. This radical scavenging activity was seen to increase with concentrations in the rice extracts, suggesting that these extracts scavenged the radical in a dose dependent manner. The results demonstrate that the IC_{50} was low for the rice variety of “karungkuravai” ($91.08 \pm 0.82 \mu\text{g/ml}$) and high for “mappilai samba” ($359.43 \pm 24.16 \mu\text{g/ml}$) suggesting that the free radical

Table 3 Total phenolic content, FRAP value and IC_{50} of rice varieties

Rice variety	TPC ($\mu\text{g GAE/g}$)	FRAP value ($\mu\text{M/mg}$)	IC_{50}^* ($\mu\text{g/ml}$)
Karungkuravai	80.25 ± 1.39^c	3181 ± 16.52^c	91.08 ± 0.82^c
Mappilai Samba	47.35 ± 1.78^d	483 ± 11.78^d	359.43 ± 24.16^d

Data's are mean \pm standard deviation ($n=3$)

Data's were statistically analysed using ANOVA and Tukey's test. Means in the same columns with different superscripts are significantly different, ($p < 0.05$)

TPC total phenolic content, FRAP ferric reducing antioxidant power, IC_{50} concentration of extract required to scavenge 50 % of free radicals

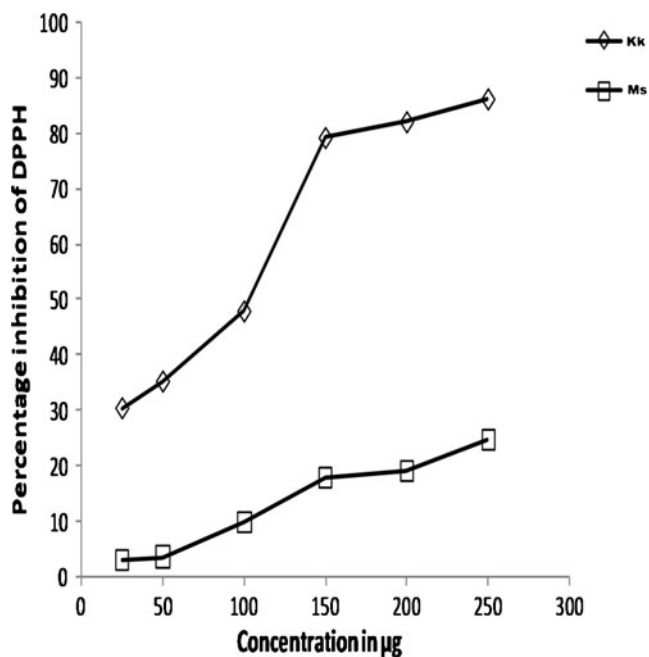


Fig. 6 DPPH radical scavenging activity (%) of **a** *Kk* Karungkuravai; and **b** *Ms* Mappilai samba rice varieties

scavenging capacity among the rice varieties is high in “karungkuravai”. IC_{50} of positive control BHT was 15.134 $\mu\text{g/ml}$. The IC_{50} value of rice varieties is shown in Table 3.

The estimation of antioxidant capacity is a stepping stone test for any plant extract for further determination of its pharmaceutical value. There is a number of methods currently in use for estimation of antioxidant activity in plants and no one method is considered a significant index to ascertain the antioxidant activity (Pavel et al. 2006). Both DPPH and FRAP assay which are most commonly used method to quantify antioxidant activity were used to determine the antioxidant potential of all four rice varieties (Ozgen et al. 2006). Both the rice varieties showed a free radical scavenging capability. The scavenging capacity of both rice varieties showed an increase with increase in their concentration thereby suggesting that the radical scavenging activity of the rice sample is dose dependant. The rice variety of “Karungkuravai” showed a higher DPPH inhibitory activity at lower concentrations when compared to other rice variety (Fig. 6). The ability of the rice extracts to reduce the ferric ions was determined using FRAP assay. This method is considered a sensitive method for estimation of antioxidant activity in biological fluids plant homogenates and pharmaceutical plant products (Vasco et al. 2008). Results of FRAP assay is also indicative that the reducing capacity of the rice extract increases with increase in concentration. FRAP value was seen to be high in the rice variety of “Karungkuravai” which also showed a high TPC value and a good DPPH scavenging capacity compared to “Mappilai Samba”. The results also indicated that increase in

Table 4 Correlation of total phenolic content with DPPH and FRAP

Rice variety	TPC vs. DPPH scavenging	TPC vs. FRAP assay
Karungkuravai	R 0.933	0.959
	R^2 0.872	0.920
	P 0.02	0.04
Mappilai Samba	R 0.982	0.993
	R^2 0.964	0.987
	P 0.017	0.006

R Pearson correlation coefficient, R^2 regression coefficients, *P* probability values, *TPC* total phenolic content, *FRAP* ferric reducing antioxidant power, *DPPH* 1, 1-diphenyl-2-picrylhydrazyl

TPC values in each rice variety showed a corresponding increase in DPPH scavenging activity and FRAP value.

Correlation analysis

The correlations between total phenolic content and antioxidant assays (DPPH and FRAP) was computed. The correlation coefficients (*R*) and coefficient of regression (R^2) were calculated for both rice varieties and are listed in Table 4. In general, the phenolic content had a strong positive correlation with both the antioxidant assay which is in accordance with previous studies (Yafang et al. 2011; Rattanachitthawat et al. 2010; Rao et al. 2010). In both rice varieties, the correlation coefficient and regression coefficient were seen to be higher between TPC and FRAP when compared to TPC and DPPH. The correlation and regression analysis showed that the TPC had a significant positive correlation with antioxidant activity of the rice suggesting that they may be responsible for the antioxidant activity seen in the rice varieties. Similar results were also seen in some earlier studies (Chi et al. 2007; Jin et al. 2009).

Conclusion

The GC MS analysis showed presence of phytosterols in high quantity in both rice varieties. Static head space analysis identified medically important molecules like curlone and 1-4-benzodiazepine-2-one present in the rice varieties, these photochemicals have been identified for the first time in any rice variety. Both rice varieties showed presence of phenols and antioxidant capacities. Phenolic content and antioxidant capacity are significantly correlated with each other. Results from the study identifies the medicinal rice variety of “karungkuravai” and “mappilai samba” as ideal candidates for further detailed research for isolation of pharmaceutically important chemicals, development of novel pharmaceutical products for alternate and safe treatment of various ailments and also in formulations of health/dietary supplements.

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Conflict of interest The authors declare that there is no conflict of interest.

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