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TOTAL PHENOLIC CONTENT AND "IN-VITRO" ANTIOXIDANT ASSAY OF TWO MEDICINAL RICE VARIETIES - KARUNGKAVUNI AND KUZHIADICHAN.

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ABSTRACT

The objective of this study is to investigate the total phenolic content and the antioxidant activity of two medicinal rice varieties of India "Kuzhiadichan" and "Karungkavuni". Total phenolic content (TPC) was estimated using Folin Ciocalteu colorimetric method, antioxidant assay was done using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay and ferric reducing antioxidant power (FRAP) assay. Phenols were found to be present in both the rice varieties. Both rice varieties also showed antioxidant activity in DPPH assay and FRAP assay, the IC 50 value of the rice varieties were 46.98 µg/ml and 28.01 µg/ml for "Kuzhiadichan" and "Karungkavuni" varieties respectively. The correlation coefficient and regression analysis of total phenolic content with DPPH assay and FRAP assay showed significant positive correlation coefficient values and coefficient of regression values.

KEYWORDS: Rice, total phenolic content, 1, 1-diphenyl-2-picrylhydrazyl, ferric reducing antioxidant power, antioxidant activity.

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1. INTRODUCTION

The presence of high levels of free radicals like reactive oxygen species (ROS) in the body is a major reason for the onset of diseases like cancer, obesity, cardiovascular disorders [1, 2]. The ROS are produced both endogenously and from exogenous sources like food, irradiation, drugs and toxins in the body [3]. The presence of antioxidants naturally produced in the body and supplied through diet play a major role in control of these ROS. The foods that are considered to be an ideally good health food should be a good source of natural antioxidants [4]. The consumption of fruits, legumes, vegetables and whole grains can supplement in the diet with natural antioxidants [5-7], besides other bioactive compounds. Phytochemicals like phenolic compounds play a crucial role in the radical scavenging activities [8, 9]. Dietary intake of polyphenols has proved to reduce chronic diseases like cancer, obesity, cardiovascular disorders[10]. 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 [11-16]. Rice is the second most consumed grain after wheat and is also the staple food of 2/3rd of the world population and therefore carries utmost importance. Apart from being a major energy source to over 2 billion people in the world , rice is also a medicine [4, 17, 18]. Rice is also a rich source of natural antioxidants [19-22, 16]. Antioxidant activity of pigmented rice and rice bran has been earlier reported [23-28]. 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 [29]. Works have been done on measuring the antioxidant potential of some of the indigenous medicinal rice [16, 30]. The present study is aimed in examining two native medicinal rice varieties of Tamil Nadu for presence and comparison of phenolic compounds and their antioxidant activity using DPPH and FRAP assay.

2. MATERIALS AND METHODS

2.1. Plant Material

Dehulled and unmilled rice samples of "Kuzhiadichan" and "Karung Kavuni" were procured from organic rice farmers from the Tanjavur ($10^0$46'56.99''N $79^0$7'52.51''E) and Tiruvarur ($10^0$46'17.76''N $79^0$38'12.48''E) Districts of Tamil Nadu in southern India.

2.2. 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.

2.3. 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 hours 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 hours**.** The extract was collected again using Whatman no. 1 filter paper and evaporated below 40°C, which was then used for further phytochemical analysis.

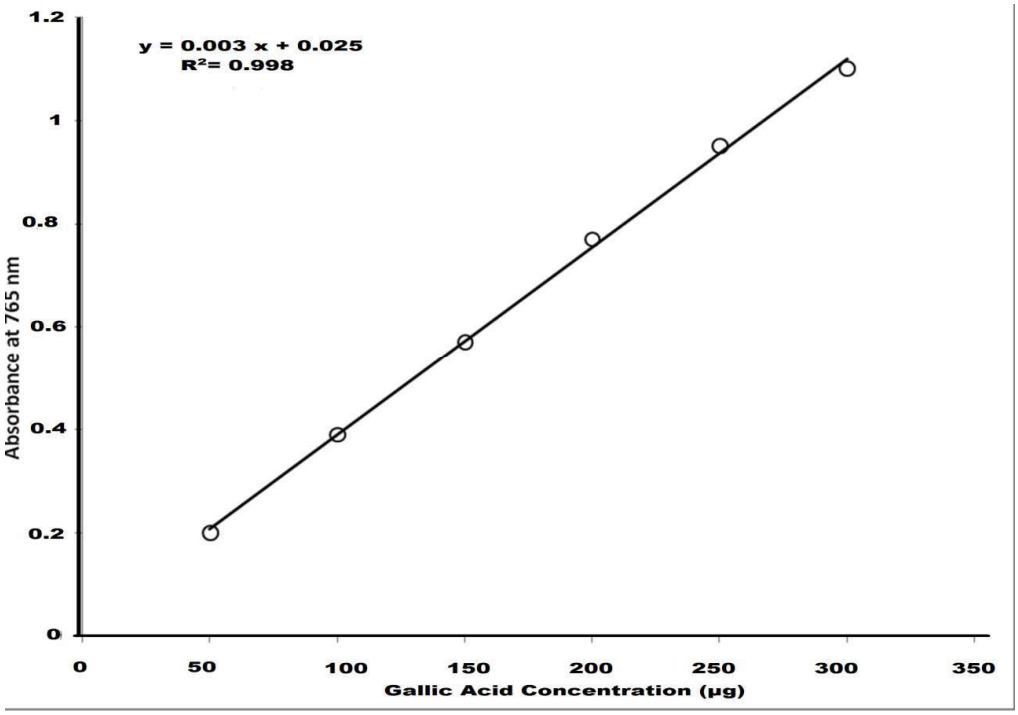
2.4. Determination of total phenolic content

The amount of phenolic compounds in the extracts was determined by the Folin Ciocalteu colorimetric method[31]. Calibration curve was prepared using Gallic acid as standard (10mg/10ml). 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 (1mg/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 minutes and was incubated for 30 minutes. 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 (Graph 1) obtained with Gallic acid as standard.

Graph 1

Standard curve to estimate Gallic acid equivalents in total phenol assay.

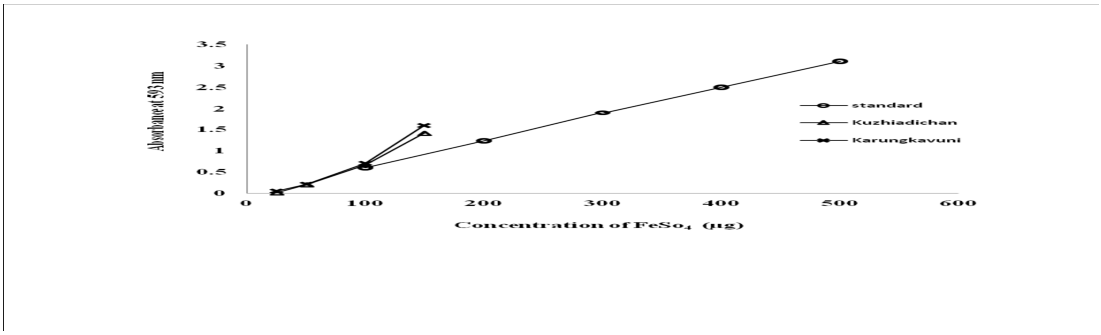


2.5. Antioxidant assay

2.5.1. FRAP assay

Antioxidant activity was measured by ferric reducing antioxidant power (FRAP) assay of Benzie and Strain (1996) [32]. FRAP assays uses antioxidants as reductant in a redox-linked colorimetric method, employing an easily reduced oxidant system present in stoichiometric excess. Ferric tripyridyl triazine (Fe III TPTZ) complex is reduced to ferrous form at low pH, which can be measured at 593 nm.The change in absorbance is 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 (300mM, pH 3.6) with TPTZ (2, 4, 6-tri (2-pyridyl)-s-triazine) (10mM in 40mM dilute HCl) and ferric chloride (20mM) in the ratio of 10:1:1. Ferrous sulphate (1mM) was used as standard. Various concentrations (25, 50, 100, 150 µg) of the sample was aliquoted from the sample extract stock (1mg/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 minutes. After incubation the absorbance was measured at 593 nm. Ferrous sulphate standard was processed in the same way and calibration curve (Graph 2) was generated using various concentrations of ferrous sulphate (100 – 500 µg). The FRAP value of the extract was calculated from the standard graph.

Graph 2
Comparative graph of absorbance of rice samples with that of standard ($FeSo_4$) in FRAP assay.



2.5.2. 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 activity according to Clemente et al [33]. DPPH is a stable free radical with purple color (absorbed at 517nm). 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 (1mg/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. Optical density was measured at 517 nm after keeping the solution mixtures in dark for 30 minutes.The optical density was recorded and % inhibition was calculated using the formula given below

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

IC50 values (concentration of extract required to scavenge 50% of free radicals) were calculated by log probit analysis [34].

2.5.3. Statistical Analysis

All the tests were performed in triplicate and values are expressed as mean ± 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. IC 50 was calculated by log probit analysis using StatPlus v2009. $P < 0.05$ was considered statistically significant.

3. RESULTS & DISCUSSION

The rice varieties of "Kuzhiadichan" and "Karungkavuni" are traditional rice varieties of Tamil Nadu in southern India. "Kuzhiadichan" is ideal for lactating mothers, as it increases the milk flow. Rice gruel of this variety is given to new mothers to impart strength. It also acts as a galactogogue when consumed by new mothers. Their rice bran is heated and used as pillow to cure common cold, cough and fever, "Karungkavuni" is a black rice grown in Tamil Nadu and is considered anti-diabetic[35].

3.1. 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 1. Significant differences were observed for TPC among the two rice varieties. For all the concentrations of sample extract, the total phenolic content of "Karungkavuni" (38.6±1.14 µg GAE/g) showed higher phenolic content compared to "Kuzhiadichan" (25.7±1.99 µg GAE/g). The present study showed the presence of phenolic compounds in both the rice varieties. Among the rice varieties tested "Karungkavuni" showed comparatively a higher phenolic content than the rice varieties of "Kuzhiadichan". In both the rice varieties increase in concentration also showed an increase in the phenolic content value. This rice variety also exhibited a high FRAP value; however the IC 50 value was comparatively higher in the rice variety of "Kuzhiadichan".

Table-1

Total phenolic content, FRAP value and IC 50 of rice varieties

Rice variety	TPC (µg GAE/g)	FRAP value (µM/mg)	IC 50* (µg/ml)
Kuzhiadichan	25.7±1.99[a]	1050±22.06[a]	46.98±0.73[a]
Karungkavuni	38.6±1.14[b]	1140±20.15[b]	28.01±0.36[a]

Data's are mean±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).*IC 50 was calculated using log probit analysis. TPC- Total phenolic content, FRAP- Ferric reducing antioxidant power, IC-50- Concentration of extract required to scavenge 50% of free radicals.

3.2. Antioxidant assay

The estimation of antioxidant capacity is a stepping stone test for any plant extract for further determination of its pharmaceutical value. There are 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[36] . Both DPPH and FRAP assay which are most commonly used method to quantify antioxidant activity was used to determine the antioxidant potential of the rice varieties [37].

3.2.1. FRAP assay

The ability of the rice extract 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[38]. Optical density (O.D.) values of rice variety "Kuzhiadichan" and "Karungkavuni" was seen to be more or less similar to that of the standard ($FeSo_4$) at 100 µM concentration, at higher concentration the O.D. values were higher than that of standard (Graph 2). FRAP value was noted to be high in the rice variety of "Karungkavuni" than. FRAP value (at 100 µg of rice sample extract) of the rice varieties is shown in Table 1. Results of FRAP assay is indicative that the reducing capacity of the rice extract increases with increase in concentration. FRAP value was seen to be highest in the rice variety of "Karungkavuni" which also showed the highest TPC value compared to "Kuzhiadichan".

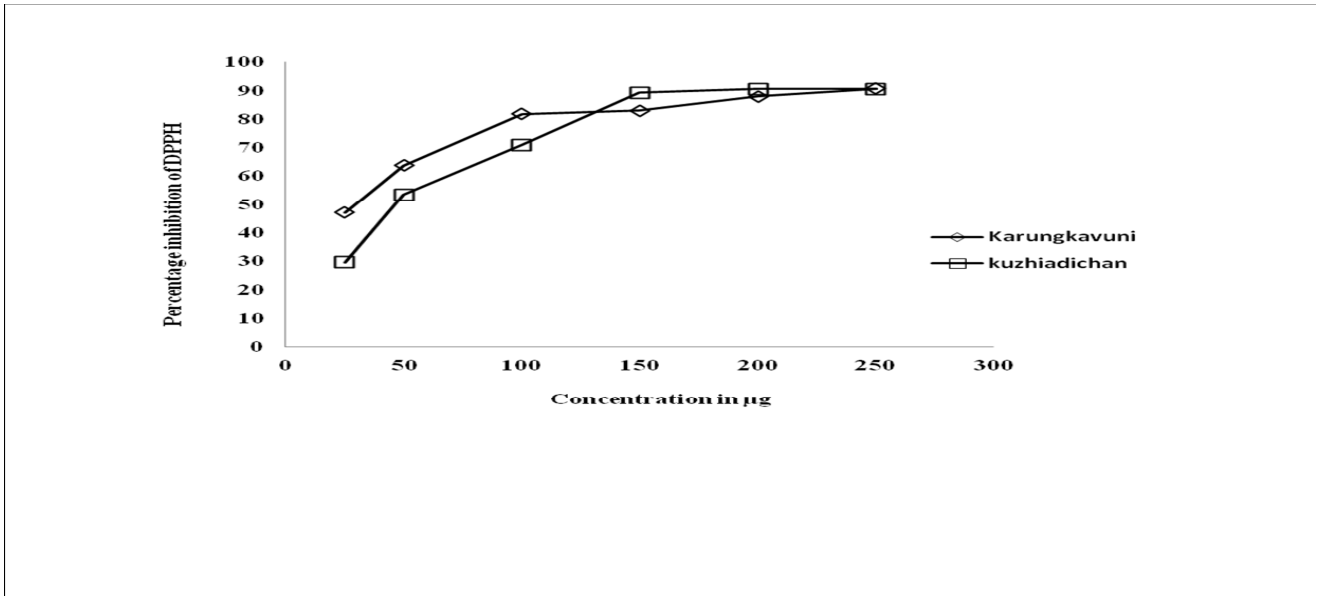
3.2.2. DPPH assay

Free radical scavenging activities of the rice sample extracts were assessed by the DPPH assay. Standard graph plotting the various concentrations of rice extracts against the percentage inhibition (Graph 3) 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 all the rice extracts, suggesting that these extracts scavenged the radical in a dose dependent manner. The results demonstrates that the IC 50 was low for the rice variety of "Karungkavuni" (28.01±0.36 µg/ml) suggesting that the free radical scavenging capacity among the two rice varieties is highest with "Karungkavuni". IC 50 of positive control BHT

was 15.134µg/ml. The IC 50 value of both the rice varieties is shown in Table 1. The DPPH results signify that both rice varieties have a strong free radical scavenging capability. The scavenging capacity of all the rice variety 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 "Karungkavuni showed a higher DPPH inhibitory activity at lower concentrations when compared to other rice variety, but at higher concentrations the inhibitory activity was seen to be almost same among the rice varieties of "Karungkavuni", "Kuzhiadichan" (Graph 3). The IC 50 concentration was seen to be low in the rice variety of "Karungkavuni" followed by "Kuzhiadichan" (Table 1).

Graph 3

DPPH radical scavenging activity (%) of different concentrations of rice extracts.



3.3. 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 all four rice varieties and is listed in Table 2. In general, the phenolic content had a strong positive correlation with both the antioxidant assay which is in accordance with previous studies [39,40,16]. In the rice variety of "Karungkavuni" the correlation coefficient and regression coefficient was seen to be higher between TPC and FRAP when compared to TPC and DPPH. The results also indicated that increase in TPC values in each rice variety showed a corresponding increase in DPPH scavenging activity and FRAP value. The correlation and regression analysis points that the Total Phenolic Content had a significant positive correlation with antioxidant activity of the rice varieties, suggesting that they may be responsible for the antioxidant activity seen in the rice variety. Similar results were also seen in some earlier studies [41, 42].

Table 2
Correlation of total phenolic content with DPPH and FRAP.

Rice variety		TPC vs. DPPH scavenging	TPC vs. FRAP assay
Kuzhiadichan	R R^2 P	0.918 0.962 0.018	0.992 0.984 0.007
Karungkavuni	R R^2 P	0.982 0.966 0.017	0.975 0.950 0.02

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.

4. CONCLUSION

This is the first report on the antioxidant activity and total phenolic content of the native medicinal rice varieties of "Kuzhiadichan" and "Karungkavuni". Results from the study suggest that these medicinal 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 "Kuzhiadichan" and "Karungkavuni" 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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