PRAJÑĀ - Journal of Pure and Applied Sciences Vol. 22 & 23: 44 - 51 (2015)
ISSN 0975 - 2595

A COMPARATIVE STUDY OF TOTAL PHENOLIC CONTENT AND ANTIOXIDANT CAPACITY OF THREE VARIETIES OF RICE *(Oryza sativa* L.*)* MAINLY CONSUMED IN GUJARAT (INDIA)

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ABSTRACT

Epidemiological studies suggest that consumption of whole grain in regular meals is desirable for health benefits beyond basic nutrition and to reduce the risks of many chronic diseases as they contain antioxidants, including phenolic compounds. Rice (*Oryza sativa* L.) is an important component of the human diet. Mainly it contains greater levels of phenolic acids, such as ferulic acid, p-coumaric and caffeic acids and other antioxidants compounds. Three different varieties of rice i.e. Gujarat 17, Parimal and Basmati were subjected to analyse their Total Phenolic Content (TPC), Flavonoid Content and Total Antioxidant Capacity (TAC) by using seven different assay methods. The highest TPC, TAC measured through FRAP and DPPH RSA was observed in Parimal variety of rice, while Gujarat 17 variety of rice showed highest SORSA (% inhibition) and ABTS RSA where as Basmati variety of rice noted to be high in Flavonoid, RPA, HRSA and lower IC50 values for NORSA. This finding implies the moderate phenolic content and antioxidant capacity in the selected rice varieties mainly consumed in Gujarat.

Keywords: *Oryza sativa* L., Total phenolic compounds, Total Antioxidant Capacity

INTRODUCTION

Diet plays an important role in the maintenance of balance between antioxidant and prooxidant. Prooxidants derived either from metabolic process or from peripheral sources [1] can potentially react with the body's own molecules [2]. If the balance between prooxidant and antioxidant is in the favor of prooxidant, that condition is known as oxidative stress. Oxidative stress may be ultimately resulting in inflammatory and/or chronic diseases, such as cardiovascular diseases (CVD), cancer, and diabetes [3-5]. Dietary enzymatic and non-enzymatic antioxidants known to neutralize free radicals or their actions by maintaining proper balance of prooxidants and antioxidants in the body [6].

According to recent dietary guidelines for Indians, cereal foods are recommended as the largest component of the daily intake as grains are put at the base of the food guide pyramid [7]. These cereals are found to be rich sources of fiber, vitamins, minerals, and phytochemicals including phenolics, carotenoids, vitamin E, lignans, β-glucan, inulin, resistant starch, sterols, and phytates [8-10]. India remains comparatively second largest white rice producer globally and rice (*Oryza sativa* L.) is the most important cereal crop as it is the staple food of around 50% of the world's population [11]. In India, rice is the major staple food for the people of southern states such as Tamilnadu, Kerala, Andhra Pradesh, Karnataka, Orissa, Chhattisgarh and Maharashtra. Besides, the people of other states including Gujarat also use rice in their daily diet. Usually people eat boiled rice with cooked pulses, vegetables, fish or meat. Furthermore, rice water is demulcent, nourishing drink in febrile diseases as well as in intestinal inflammation [12].`

Rice is a good source of antioxidants, including phenolic compounds particularly ferulic, p-coumaric, syringic, and isoferulic acids are present as bound forms [13] which are not present in significant quantities in fruits and vegetables [14-15]. Ferulic acid which is the major hydroxycinnamic acid derivative accounting major component of the plant cell wall [16] and it has been known as an antioxidant which is effective toward anti-inflammation and inhibition of tumour initiation and as a preservative [17]. p-Coumaric acid, another hydroxycinnamic acid, is a precursor of 4-coumaroyl-CoA which serves as a substrate to form the basic skeleton of all flavonoid derivatives [16]. ɣ-Oryzanol (or steryl ferulate esters) may contribute more to the reduction of cholesterol oxidation [18] and is a powerful inhibitor of iron driven hydroxyl radical formation, which is usually considered to be the major antioxidant present in less amount in polished rice compared to rice bran.

Traditionally in India, rice is used as polished rice and consumed as cooked rice as well as in the form of puffed rice, rice flakes and fermented preparations like *idli*, *dosa* and many other forms. Although a number of rice varieties have been commercially cultivated in India, research on the said promising rice varieties remains limited. Considering this, objective of the study was to evaluate the total phenolic content, flavonoid content and total antioxidant capacity by seven different assay methods in three commercial rice varieties namely Gujarat-17, Parimal and Basmati.

MATERIALS AND METHODS

Chemicals: L-Ascorbic Acid (95210), DPPH (2,2-Diphenyl-1-picrylhydrazyl) (D 9132), Gallic acid (G 7384), N-(1-naphthyl ethylenediamine dihydrochloride) (N 9125), 1,10-Phenanthroline(320056), Rutin hydrate (R 5143), Sulphanilamide (33626), TPTZ (2,4,6 –Tris (2-pyridyl)–s–triazine) (T 1253), TAPS {3-[Tris-9-hydroxymethyl methylamino] propane sulfonic acid}(T 5130), Diethylnetriamine penta acetic acid (D1133) and Pyrogallol (254002) were purchased from Sigma Aldrich Company. Sodium Nitroprusside (dihydrate) (71778) and

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Trolox (6-Hydroxy-2, 5, 7, 8 – tetra methylchromane-2-carboxylic acid) (56510) were of Fluka brand purchased through a local dealer.

Sample collection and extraction: Commercially available Gujarat-17, Parimal and Basmati rice harvested in the year of 2012 were purchased from the local market of Anand (Gujarat, India). These rice samples of 250gms each were procured from four different shops, pooled, sieved, cleaned, ground to flour using Kitchen grinder and sieved to a fine powder. From that flour, duplicate extract of each sample was made using 80% methanol (pH-2) and stored at -20°C for the determination of TPC and TAC.

Determination of Total Phenols:

Total Phenolic Content: Total phenolic content of methanolic extract of rice was determined using Folin – Ciocalteu method and the absorbance was read at 750 nm [19]. Gallic acid solution of different concentrations treated same as the sample, the straight line equation was generated from the standards and the results were expressed as milligrams of gallic acid equivalent per 100g of rice (mg GAE/100g).

Flavonoid Content: Concentrated extracts of rice was used for the estimation of Flavonoid content and the absorbance was read at 510nm [20]. Comparison was done using the rutin as standard and the results were expressed as milligrams of rutin equivalent (mg RE) per 100 g of rice.

Determination of Total Antioxidant Capacity:

Ferric Reducing Antioxidant Power (FRAP): The FRAP assay was carried out by following the method developed by Benzie and Strain [21]. Trolox standard curve was developed using different aliquots and results were expressed in terms of mg Trolox Equivalent Antioxidant Capacity (TEAC) per 100 g of rice.

Reducing Power Assay (RPA): This assay was performed using the method described by Oyaizu [22]. Different aliquots of Trolox were treated as standard and results were expressed in terms of TEAC per 100 g of rice (mg TEAC/100g).

DPPH Radical Scavenging Activity: The method described by Brand-Williams et al. [23] was followed for determining the antioxidant activity of the methanolic extract of rice, on the basis of the scavenging activity of the stable 2, 2- Diphenyl-1-picrylhydrazyl (DPPH) free radicals. Extract was added to methanolic solution of DPPH and absorbance at 517 nm was recorded after 20 minutes. Percentage inhibitions were calculated using the formula (1):

(Acontrol – Asample/Acontrol)*100 and the results were expressed in terms of TEAC per 100 g of rice (mg TEAC/100g).

ABTS Radical Scavenging Activity: The ABTS (2, 2'-Azino-bis (3–ethyl benzothiazoline-6-sulfonic acid) diammonium salt) radical scavenging activity assay was carried out using an autobleaching method [24] with little modification. The percentage inhibition was calculated using the formula (1) and the results were expressed in terms of TEAC per 100 g of rice (mg TEAC/100g).

Nitric oxide Radical Scavenging Activity: Nitric oxide was generated from sodium nitroprusside and measured as described by Greiss reaction [25] with some modifications [26]. IC_{50} values (concentration of extract required to scavenge 50 percent free radical) were calculated from the regression equations prepared from the concentrations of the extracts. Percentage nitric oxide radical scavenging activity (NO RSA) was calculated using the formula (2): [1- (Acontrol – Asample/Acontrol)] *100.

Hydroxyl Radical Scavenging Activity: The hydroxyl radical-scavenging activity of samples was measured [27] with some modifications [28]. In this system, hydroxyl radicals were generated by the Fenton reaction. Hydroxyl radicals could oxidize Fe2+ into Fe3+, and only Fe2+ could be combined with 1, 10-phenanthroline to form a red compound (1,10-phenanthroline-Fe2+) with the maximum absorbance at 536 nm. The reaction mixture without any antioxidant was used as the negative control, and without H2O2 was used as the blank. The hydroxyl radical scavenging activity (HRSA) was calculated by the following formula (3): HRSA (%) = [(As-Ac)/ (Ab-Ac)] × 100. Where As, Ac, and Ab were the absorbance values of the sample, the control and the blank after reaction, respectively.

Superoxide Radical Scavenging Activity: The superoxide radical scavenging activity was assayed by following the method of the monitoring of the inhibition of pyrogallol autoxidation [29-30]. This assay is dependent on the reducing activity of test compound by an O2-. dependent reaction, which releases chromophoric products. The absorption was read at 320nm using UV Visible spectrophotometer (Shimadzu Inc., Kyoto, Japan). The superoxide radical scavenging activity (SO RSA) was determined as the percentage of inhibiting pyrogallol autoxidation, which was calculated using the formula (1) from absorbance in the presence or absence of methanolic extracts of rice.

Statistical analysis: The experimental results are expressed as mean ± standard deviation (SD) of four measurements of each sample. Afterwards, the results were subjected to Analysis of variance (ANOVA), the significance of mean differences was determined by Duncans' post hoc test considering $p \leq 0.05$ significant level of difference. The Pearson's correlation coefficients (R) were also calculated to establish relationships among data obtained. All the statistical calculations were done using SPSS version 17.0.

RESULTS AND DISCUSSION

As mentioned earlier that rice is a staple cereal used in India and Gujarat. Many studies reported the TPC and TAC of rice. Total phenolic content and antioxidant capacity by different assays of Indian varieties of red/brown and white whole grain rice has been reported by Biswas et al. [31]. A review of literature reveals that ten high yielding rice varieties of Bangladesh [32], brown and

polished rice varieties of Thailand [33], milled fractions of different *indica* rice varieties cultivated in southern China [34], two medicinal rice varieties of India [35] and some commercial rice varieties in Taiwan [36] have been analysed for their phenolic content and bioactive constituents. However, Gujarat 17, Parimal and Basmati varieties of rice have not been extensively studied.

The total phenolic content (TPC) of rice varieties ranged from 47.2 to 52.5 mg GAE/100g and ranked as Gujarat 17 ≈ Basmati < Parimal (Figure 1). Similar findings were reported by Sreeramulu et al. [37] as they extracted rice powder using 60% acidic methanol and found 47.64 mg GAE/100g. Almost similar results phenomenon was recorded in the endosperm fraction of polished *indica* rice varieties cultivated in southern China [34] and in milled white rice [38]. Biswas et al. [31] extracted powdered rice in 70% methanol and found much higher phenolic content in Indian varieties of white rice as compared to purple/red/brown rice. On the other hand, various studies are reported to have lower TPC in different rice varieties [32, 33, 36].

Significantly higher ($p \leq 0.05$) flavonoid content was observed in Basmati variety of rice i.e. 39.9 ± 1.92 mg RE/100g followed by Parimal (27.3 ± 3.54 mg RE/100g) and Gujarat 17 (20.2 ± 0.95 mg RE/100g) variety of rice (Figure 1). Huang and Ng [36] found flavonoid ranging from 14 to 106 mg quercetin eq. / 100g in non-pigmented polished rice varieties of Taiwan. The higher flavonoid contents were found by Ti et al. [34] who extracted rice in 95% chilled acidified methanol and by Shen et al. [39] who extracted dehusked white rice flour in methanol containing 1% HCl. However, Vichapong et al. [33] reported lower values of total flavonoid content in polished rice varieties.

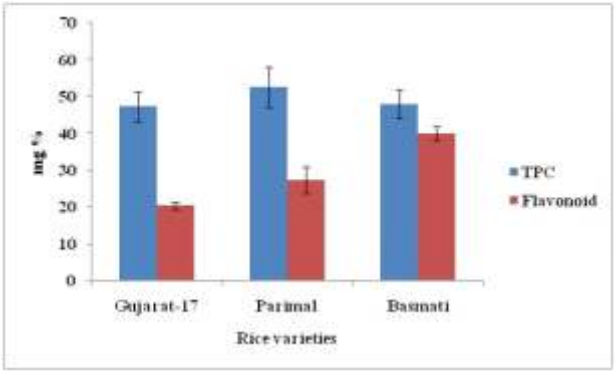

Figure. 1. Total phenolic and flavonoid content of selected rice varieties

The ability of the methanolic rice extracts to reduce the ferric ions was determined using ferric reducing antioxidant assay and it is considered as a sensitive method for estimation of antioxidant activity in biological fluids, plant homogenates and pharmaceutical plant products [40]. Table 1 represents the total antioxidant capacity measured by FRAP, RPA, DPPH RSA and ABTS RSA. The TAC measured by FRAP was noted to be high in Parimal (35.5 mg TEAC/100g) variety of rice and ranked as Parimal ≈ Gujarat 17 > Basmati. Similar results were observed for FRAP values of different rice varieties of Bangladesh and these values were ranged between 90.22 to 195.78 µM AAE/100g (i.e. 15.89 to 34.48 mg AAE/ 100g) [32]. Comparatively higher FRAP values were also reported in some studies [34, 37, 38].

Table 1 Total antioxidant capacity (TAC) by FRAP, RPA, DPPH RSA and ABTS RSA of selected rice varieties

	Total Antioxidant Capacity (TAC) (mg TEAC/100g)			
Rice varieties	**FRAP**	**RPA**	**DPPH RSA**	**ABTS RSA**
Gujarat-17	34.49 ± 3.31 [b]	88.27 ± 7.57 [a]	665.53 ± 27.59 [a]	1.23 ± 0.15 [b]
Parimal	35.5 ± 3.89 [b]	95.4 ± 9.00 [a]	690.75 ± 10.38 [a]	1.05 ± 0.13 [b]
Basmati	29.11 ± 3.78 [a]	96.5 ± 7.03 [a]	678.9 ± 9.26 [a]	-[$a]

Values are mean ± S.D of four observations. Mean values with the different superscripts within a column are significantly different from each other ($p \leq 0.05$).
[$] indicates below the level of detection.

The reducing power measures the Fe^{3+}–Fe^{2+} transformation which was investigated in the presence of methanolic extract of rice. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging [41,42]. Increased in absorbance of methanolic extract of rice varieties showed increased reducing power. It was found to be 96.5, 95.4 and 88.27 mg TEAC/100g of Basmati, Parimal and Gujarat 17 variety of rice respectively. 79 mg AAE/100g RPA value of white rice was reported by Sreeramulu et al. [37] which is comparatively lower than that of present study. Biswas et al. [31] observed higher reducing power in white rice varieties than in the red rice varieties.

The free radical chain reaction is broadly established as a general mechanism of lipid peroxidation. Radical scavengers may directly react with and quench peroxide radicals to terminate the peroxidation chain reaction and enhance the quality and stability of food products [43]. Free radical scavenging activities of the rice varieties were assessed by DPPH radical scavenging activity. In the presence of hydrogen donating antioxidant, stable 2, 2- Diphenyl-1-picrylhydrazyl (DPPH) free radicals reduced to the yellow coloured non-radical form DPPH–H [23]. The DPPH RSA of currently tested rice varieties showed following order: Parimal (690.75 mg TEAC/100g) > Basmati (678.9 mg TEAC/100g) > Gujarat 17 (665.53 mg TEAC/100g). Vichapong et al. [33] and Sreeramulu et al. [37] measured DPPH RSA and found 6.5 to7.91mg AAE/100g and 123 mg TE/100g respectively. Thus these results indicate that increase in TPC values in methanolic extract of each rice variety

leading to a corresponding increase in FRAP value and DPPH scavenging activity. Similar trend was observed in a recent study [35] for an *in-vitro* assay of antioxidant activity of two medicinal rice varieties also.

The ABTS/$K_2S_2O_8$ system used to generate blue-green radical cation $ABTS^{+}$. Bleaching of a preformed solution of radical cation $ABTS^{+}$ has been extensively used to evaluate the antioxidant capacity of complex mixtures and individual compounds where the scission of the peroxodisulfate could take place after the electron transfer. $ABTS^{+}$ assay is operable over a wide range of pH, is inexpensive, and more rapid than that of DPPH assay [44,45]. Gujarat 17 and Parimal rice variety showed approximately similar values for ABTS RSA i.e. 1.23 mg TEAC/100g and 1.05 mg TEAC/100g respectively where as Basmati rice did not show ABTS RSA, which may be due to the below level of detection. Higher ABTS RSA i.e. 0.196 mM TEAC/100g (49.1 mg TEAC/100g) was reported by Shen et al. [39].

It is well known that the experimental results may influenced by radical system used for antioxidant evaluation, and two or more radical systems are required to investigate the radical-scavenging capacities of a selected antioxidant [46]. Reactive oxygen intermediates (ROIs) are partially reduced forms of environmental oxygen (O_2). They commonly come about because of the excitation of O_2 to form singlet oxygen (O_2^1) or from the transfer of one, two or three electrons to O_2 to form, respectively, a superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) or a hydroxyl radical (HO^-). In contrast to atmospheric oxygen, ROIs are capable of unrestricted oxidation of various cellular components and can lead to the oxidative destruction of cell [47-50]. Accounting to the importance, Nitric oxide, Hydroxyl and Superoxide radical scavenging activity of rice varieties were analysed in the present study and the results are depicted in Table 2.

Table 2 IC_{50} values of Nitric oxide radical scavenging activity, % Hydroxyl and Superoxide radical scavenging activity of selected rice varieties

Rice varieties	IC_{50} values of NO RSA (mg)	HRSA (%)	SO RSA (%)
Gujarat-17	25.6 ^{b}	13.74 ± 0.90^{b}	31.25 ± 1.38 ^{b}
Parimal	36.7 ^{c}	11.90 ± 1.26^{a}	29.86 ± 4.16 ^{b}
Basmati	22.6 ^{a}	16.51 ± 0.70^{c}	16.32 ± 1.33 ^{a}

Values are mean ± S.D of four observations. Mean values with the different superscripts within a column are significantly different from each other ($p \leq 0.05$).

Several physiological processes like neural signal transmission, immune response, control vasodilatation and control of blood pressure etc. required an essential bioregulatory nitric oxide molecule [51-54]. Moreover, the elevation of NO results in several pathological conditions, including cancer [55]. The % NO RSA and IC 50 values for rice varieties were analysed graphically and presented here. The ability to scavenge $NO^{\cdot}$ radical by rice varieties displayed in the order of Basmati > Gujarat 17 > Parimal rice. The NO RSA varied from 18% to 79.2% (R^2= 0.999) of Basmati, 15.7% to 69.8% (R^2= 0.971) of Gujarat 17 and 14.4% to 51.3% (R^2= 0.818) of Parimal rice variety for different concentrations of rice extract. Inhibitory concentration (IC 50), which is the half maximal effective concentration at which the NO radicals were scavenged by 50% was determined graphically by plotting the various concentrations of rice extracts against the percentage inhibition, demonstrates a significant decrease in the concentration of NO radical due to scavenging ability of the rice extracts (Figure 2). The lower IC 50 value was observed for the rice variety of Basmati (22.6 mg) followed by Gujarat 17 (25.6 mg) and high for Parimal (36.7 mg) rice. The lower IC 50 value in Basmati rice might be indicating higher free radical scavenging activity as compared to other presently studied variety of rice. This finding supports the data from the previous study [35] where the lower IC 50 value for DPPH assay demonstrating higher free radical scavenging capacity among the rice varieties.

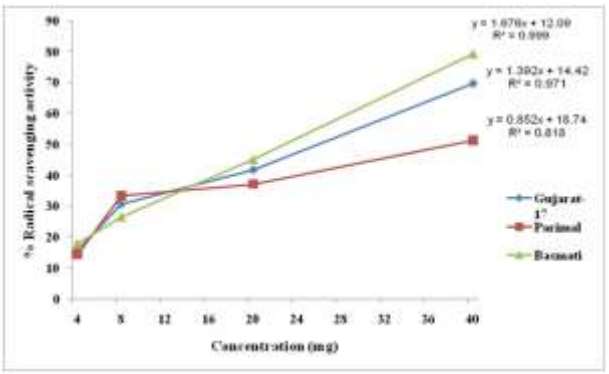

Figure 2. Nitric oxide radical scavenging activity (%) of selected rice varieties

The hydroxyl radical is one of representative reactive oxygen species generated in the body. Hydroxyl radical is generated *in vitro* by mixing iron (II) sulphate which generates ferrous ion (Fe^{+2}) with hydrogen peroxide and 1, 10-phenanthroline. Since phenanthroline- Fe^{+2} is commonly used as an indicator of redox reactions the 1, 10-phenanthroline was used in the present study. The fenton reaction with phenanthroline- Fe^{+2} complex oxidise to Fe^{+3} and produces hydroxyl radical in H_2O_2/ Fe^{+2} system [56]. Basmati rice showed highest hydroxyl radical scavenging activity (16.51%) followed by Gujarat 17 (13.74%) and Parimal (11.90%) varieties of rice, which is in contrast to the results of the TPC, TAC by FRAP and DPPH RSA, on the other hand, as similar to the results of the NORSA. A recent study [32] showed 1.37 to 5.62% HRSA for *aman* rice group and 2.0 to 5.16% HRSA for *boro* rice group and this may be due to the presence of phenolic compounds such as ferulic acid product in sample extract contain phenolic hydroxyl group, with the ability to accept electrons, which can combine with free radical competitively to decrease hydroxyl radical [57].

The principle of the pyrogallol autoxidation method is that the pyrogallol can autooxidise in alkaline solutions to produce $\cdot O^{2-}$ anion radicals; detected by a spectrophotometer, so the absorbance reflects the generation of both purpurogallin and superoxide radicals ($\cdot O^{2-}$). Obviously, a lower absorbance indicates higher inhibition of $\cdot O^{2-}$ [29]. Interestingly, highest superoxide radical scavenging activity (%) was noticed in Gujarat 17 rice variety with the value of 31.25% which is nearly double than that of Basmati rice (16.32%) and displayed significantly lower value where as Parimal variety of rice had 29.86% SO RSA, which is in contrast to the results of the TPC, flavonoid content and different TAC assays by FRAP, RPA, DPPH RSA, NORSA and HRSA. One of the possible reasons for this variation could be the difference in the mechanism of action of these methods [58].

Pearson's Correlation Coefficient (R) between total phenolic content, flavonoid content and different antioxidant capacity assays was analysed and are listed in Table 3. No correlation was found between TPC, flavonoid content and seven parameters of TAC which may be due to their excellent antioxidant activity. This reveals that antioxidant activity may be contributed by the presence of phenolic acids, especially ferulic acid, p-coumaric acid and diferulates and flavonoid compounds. Findings reported from the previous studies [31, 32, 37, 59] supports the result of the present study as they did not find any correlation between TPC and the antioxidant activities, one of the possible reason may be the synergistic effect of the components present in the methanolic extract of rice that determines the antioxidant effect of the extract [31]. The flavonoid content had a strong and negative correlation with the ABTS RSA (R= -0.839, $p \leqslant 0.01$) and SORSA (R= -0.898, $p \leqslant 0.01$). TAC measured by FRAP showed a negative and significant correlation with HRSA (R= -0.619, $p \leqslant 0.05$), but not with the other parameters measured in the present study. The positive and significantly strong correlation between ABTS RSA and SORSA (R= 0.908, $p \leqslant 0.01$) was found as well as significant and negative correlation was found between ABTS RSA with NO RSA (R= -0.626, $p \leqslant 0.05$) and HRSA (R= -0.768, $p \leqslant 0.01$). The correlation between NORSA and HRSA (R= 0.605, $p \leqslant 0.05$) was found to be positive and significant where as HRSA and SORSA showed negative and significant correlation (R= -0.767, $p \leqslant 0.01$).The negative correlations between TPC, flavonoid content and antioxidant capacity were also reported by previous studies [39, 60].

The evaluation of total antioxidant capacity which is a non-nutrient bioactive component analysis is a steppingstone test for any plant extract for further determination of its pharmaceutical value [61]. The difference in TPC, flavonoid content and total antioxidant capacity of three different rice varieties may possibly due to the presence of higher or lower concentration of particular phenolic or flavonoid compound. The difference in the results obtained in the present study and the results reported by other researchers could be due to various geographical conditions like soil, growing season, rice variety, degree of maturity [62], fertilizers, irrigation, freshness, degree of polishing etc. According to Kraus et al. [63] variations in the antioxidant systems may be due to genotypes respond differentially to stresses. In the present study, we have evaluated antioxidant behaviour of three different rice varieties mainly consumed by the people of Gujarat region of India.

CONCLUSION

Based on the data obtained from the present study concluded that rice contributes low amount of TPC but it provides ferulic acid which may not present in fruits and vegetables, which are major contributor for providing phenolic compounds to our body.

ACKNOWLEDGEMENT

We thank University Grant Commission, New Delhi, for financial support under Major Research Project and for awarding Basic Scientific Research Fellowship to the first author of this research article.

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Table 3 Correlation between total phenol, flavonoid content and total antioxidant capacity by different methods of selected rice varieties

	TPC	Flavonoid	FRAP	RPA	DPPH RSA	ABTS RSA	NO RSA	HRSA
Flavonoid	0.060							
FRAP	0.258	-0.300						
RPA	-0.229	0.469	-0.067					
DPPH RSA	0.285	0.241	0.230	0.004				
ABTS RSA	0.155	-0.839**	0.489	-0.261	-0.148			
NO RSA	-0.199	0.335	-0.464	0.049	0.018	-0.626*		
HRSA	-0.420	0.512	-0.619*	0.199	-0.404	-0.768**	0.605*	
SO RSA	-0.053	-0.898**	0.496	-0.270	0.011	0.908**	-0.554	-0.767**

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

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