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# Characterizing the pigmented traditional rice cultivars grown in temperate regions of Kashmir (India) for free and bound phenolics compounds and *in vitro* antioxidant properties

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ABSTRACT

The purpose of the research was to identify the phenolic and flavonoid compounds of seven different traditional pigmented whole rice cultivars grown in the temperate regions of Kashmir so as to study their relationship with *in vitro* antioxidant capacities. The completely pigmented rice cultivars were found to have higher phenolic, flavonoid, anthocyanin contents and exhibited higher antioxidant capacities than the light colored and sparely colored rice cultivars. A total of 40 compounds had been identified in the analyzed rice cultivars that were found to be distributed in 6 major categories with 6-phenolics, 6-flavonoids, 11-hydroxycinnamic acid derivatives, 7-hydroxybenzoic acid derivatives, 3-anthocyanins and 7-flavonoid glucosides of different flavonoid compounds. Among the free and bound fractions for each cultivars the light and sparsely colored depicted higher content of phenolics and *in vitro* antioxidant properties in bound faction, while the completely pigmented cultivars showed higher antioxidant properties in free fractions. The anthocyanins quercetin-3-*O*-galactoside, cyanidin-3-*O*-rutinoside and pelargonidin-3-*O*-diglucoside had been identified by LC-MS existing in the free fractions of the analyzed rice cultivars whereas, the free fraction of acetone + $H_2O$ possessed higher percentage of phenolic compounds as compared to methanolic extracts and bound fractions. The black colored cultivars possessed higher DPPH scavenging activity and lipid peroxidation inhibition.



## 1. Introduction

Rice (*Oryza sativa* L.) is an essential cereal crop consumed by millions of people both as a staple food as well as processed products. Rice ranks second in consumption after wheat throughout the world. Among the rice varieties pigmented rice varieties have gained much popularity in almost every country due to the presence of flavonoids and antioxidant compounds. Pigmented rice is characterized by having red, brown, black, or dark purple color in its bran layers. These compounds, located mostly in the aleuronic layers of rice kernels are especially a mixture of anthocyanin compounds that belong to the family of flavonoids (Yawadio et al., 2007). The anthocyanins present in the pigmented rice have been reported to be the main source of phenolic compounds, which are having antioxidative activities (Tabart et al., 2009). Researchers have shown great interest in exploiting the antioxidant properties and polyphenols in different rice varieties due to their diverse biological activities. The phenolic compounds identified in pigmented rice include ferulic acid, diferulates, anthocyanins, pelargonidin-3-glucoside, anthocyanidins, cyanidin-3-glucoside, and polymeric proanthocyanidin that have been associated with several health benefits like aldose reductase inhibitory activities and thus prevents the body from diabetic complications (Chun et al., 2005). The phenolic and flavonoid compounds in pigmented rice cultivars have been reported to be potent antioxidants that have the ability to scavenge free radical and singlet oxygen ions and hence prevent oxidative damage to cell constituents (Saikia et al., 2012). Also the pigmented rice cultivars have been reported to reduce oxidative stress coupled with anti-inflammation and anti-atherosclerotic lesions properties (Xia et al., 2003).

The research has been carried out to analyze the majority of the traditionally grown rice cultivars of Kashmir (India) for the presence of various bioactive components using suitable identification

*Abbreviations:* GAE, Gallic acid equivalents; RU, Rutin; TFC, Total flavonoid content; TPC, Total phenolic content; TBARS, Thiobarbituric acid-reactive species; DPPH, 2, 2 diphenyl 2 picryl hydrazyl hydrate.

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| Compounds identified | | | |
|---|---|---|---|
| 1 | (*E*)-coniferaldehyde | 20 | Ellagic acid |
| 2 | (*epi*) catechin | 21 | Ellagic acid deoxyhexoside |
| 3 | (*Z*)-ferulic acid | 22 | Ferulic acid |
| 4 | 1-*O*-caffeoylquinic acid | 23 | Gallic acid |
| 5 | 3-*O*-*p*-Coumaroylquinic acid | 24 | Hydroxybenzoic acid-*O*-hexoside |
| 6 | 4-*O*-caffeoylquinic acid | 25 | hydroxybenzoylhexsose |
| 7 | 5- pyranopelargonidin- 3-O-glucoside | 26 | Luteolin |
| 8 | 5-*O*-feruloylquinic acid | 27 | Luteolin-7-*O*-glucoside |
| 9 | Apigenin | 28 | Myrecitin |
| 10 | Apigenin-7-*O*-glucoside | 29 | p-Coumaric acid |
| 11 | Caffeic acid | 30 | pelargonidin-3-O-diglucoside |
| 12 | Caffeic acid hexoside | 31 | p-hydroxybenzoic acid |
| 13 | Caffeoyl-coumaroyl-quinic acid | 32 | Procyanidin $B_1$ |
| 14 | Carnosic acid | 33 | Protocatechuic acid |
| 15 | Chlorogenic acid | 34 | Quercetin hexoside. |
| 16 | Coumaroylquinic acid | 35 | Quercetin-3-*O*-rhamnoside |
| 17 | Cyanidin-3-*O*-rutinoside | 36 | Quercetinuronic Acid |
| 18 | dihydrogallic acid derivative | 37 | Syringaldehyde |
| 19 | Dihydroxybenzoic acid-*O*-pentoside | 38 | Syringic acid |
| | | 39 | Tricaffeoyl-hydroxyferulic acid |
| | | 40 | Vanillic acid |

and quantification technique such as LC-MS coupled with their antioxidant capacities measurement based on O/R potential and spectrophometeric analysis. The purpose of analyzing the free and bound fractions of phenolic compounds is associated with the variations in the yields of phenolic contents in different extracting solvents which could be attributed to the differences in polarities of different compounds present in the rice cultivars. The aqueous solvents had been found to be suitable for extracting some bioactive compounds with strong polarity. Acetone plus water solvent is reported as the best solvent for extraction of polyphenols with a broad range of polarity. The novelty of the present research was to assess the potential antioxidant property of these different rice cultivars in order to determine the genetic variability of these components.

## 2. Materials and methods

### 2.1. Whole rice sample preparation

Seven different traditional rice cultivars were selected for the research purpose. These included three red colored (*Zag, Kaw quder, Shel kew*), black colored *Samarkand*, one light colored blackish *Kaw kareed* and two sparsely colored *Gull zag* and *Teli zag* having some kernels with red pericarp distributed sparsely in the whole rice. The cleaned paddy grains were subjected to milling using lab rubber dehusker (Agrosa India, Pvt. Ltd) to remove the husk. The brown rice was then milled in a pilot scale grinding mill (Agrosa India, Pvt. Ltd.), passed through 60 mesh sieve to ensure uniform particle size of flour. The ground samples were stored in air tight containers at around 5 °C until analyzed. All the experiments were conducted in triplicate, unless otherwise stated.

### 2.2. Extraction of free and bound polyphenolic compounds

The flours of rice cultivars (1 g, each) were extracted with 10 ml of methanol for 15 min by vigorous mixing through vortex mixer at room temperature. The supernatant was separated by filtering through a vacuum pump and the extraction was repeated thrice. All the supernatants were then combined. The remaining residue was re-extracted by the addition of 10 ml of acetone/water solvent in the ratio of 70:30 (v/v) basis followed by recovering of supernatant as mentioned above. This extraction process was repeated and the supernatants were combined. The extracts prepared individually from both methanol and acetone/water solvents included the free compounds fractions. The final leftover residue was then used for extraction of phytochemical compounds that were covalently linked to cellular components. For this, the residue was digested with 20 ml of 2 M NaOH at room temperature for 1 h. The extract was then adjusted to pH 3 with HCl. The compounds released upon hydrolysis were extracted with 20 ml of ethyl acetate. After centrifugation at 3000 rpm for 10 min, the supernatant was collected and the extraction was repeated. After collecting the supernatants, the solvent was then evaporated under vacuum and the bounded fractions were recovered by diluting them in methanol and analyzed for their antioxidant activity.

### 2.3. Liquid chromatography-mass spectrometry (LC-MS) assay

Polyphenolic compounds in the whole grains of different rice cultivars were estimated by means of LC-MS method. All the free and bound extracts were filtered though a 0.45-$\mu$m pore size syringe-driven filter before injection. A 20–$\mu$ml of the extract solution of the rice samples were separated using a Shimadzu HPLC system equipped with a diode array detector on a 150 mm × 4.6 mm i.d., 5-$\mu$m, Cosmosil 5C18-MS-II, C18-ODS analytical column (Waters). The mobile phase included acetonitrile and double distilled water having 0.1% trifluoro acetic acid (TFA) maintained at a flow rate of 0.8 ml/min. The gradient elution was done in the following manner that is from 0 to 5 min, linear gradient from 5 to 9% solvent acetonitrile; from 5 to 15 min, 9% solvent acetonitrile; from 15 to 22 min, linear gradient from 9 to 11% solvent acetonitrile; and from 22 to 35 min, linear gradient from 11 to 18% solvent acetonitrile. Column temperature was set at 40 °C. Hydroxy benzoic acid compounds were detected at a wavelength of 280 nm where as the hydroxycinnamic acid compounds were identified at 325 nm. Phenolic compounds in the whole rice samples were identified by comparing their Retention time (Rt) and (m/z) values with UV–vis spectra with authentic