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K Rajeswari
Senior Research Fellows, Indian Institute of Millets Research, Rajendranagar, Hyderabad, Telangana, India

E Kiranmai
Senior Research Fellows, Indian Institute of Millets Research, Rajendranagar, Hyderabad, Telangana, India

Sukreeti Sharma
Senior Research Fellows, Indian Institute of Millets Research, Rajendranagar, Hyderabad, Telangana, India

Correspondence
K Rajeswari
Senior Research Fellows, Indian Institute of Millets Research, Rajendranagar, Hyderabad, Telangana, India

Preliminary screening of phytochemical content of different cereals grown in different states of India

K Rajeswari, E Kiranmai and Sukreeti Sharma

Abstract
Most of the herbal medicines are plant origin. The coloured grains have its own unique potential health characteristics due to presence of bioactive pigments. Cereals are major staple food crops in most of the developing countries. Nearly 95% of global rice production is done in Asian countries, and about half of the world's population consumes it. Some speciality rice's are not commonly consumed but possess antioxidant properties, especially the coloured varieties. The present study was designed to evaluate relative contribution of different phytochemicals in various extracts of coloured cereals like black rice, brown rice and finger millet. Phytochemical analysis results of the cereals showed the presence of all the phytochemicals in black rice variety, 'Kavuni rice' which is grown in Tamil Nadu compared to other 3 grains. Qualitative phytochemical analysis of the cereals confirms the presence of various phytochemicals like alkaloids, flavonoids, tannins, terpenoids, steroids, and glycosides in their methanolic extract than the aqueous extracts. In view of this Black rice has the great interest in pharmaceutical companies and food companies for new drugs and medicinal value added food products for curing of various diseases.

Keywords: Black rice, finger millet, brown rice, phytochemical, qualitative, analysis

Introduction
Cereal is a grain or edible seed of the grass family, Gramineae[1]. There are many different types of cereals grown worldwide, each sharing some structural similarities. Cereals have a long history of use since the beginning of civilization[2]. Many of cereals have been staple foods directly for humans and animals indirectly via livestock feed.

Cereals are the most important sources for energy and protein intake of the world population[3]. Cereals are also rich sources for B vitamins and minerals contained a range of micronutrients such as vitamin E, some of the B vitamins, magnesium and zinc. The health-promoting bioactive substances, often referred as phytochemicals are also exist in cereals[4].

Rice is the most important cereal crops, accounting for over 50% of the world's cereal production. Though there is a huge number of rice varieties (~100 000) exist only a few of them are grown widely and used.

Black rice is one known as forbidden rice or emperor's rice. This rice consists of several varieties and has a long history of cultivation in Southeast Asian countries such as India, Thailand, and China. Black rice is actually dark black in colour but once cooked it becomes deep purple in colour. It is mainly used for garnishing in different types of foods. Black rice gives impressive health benefits when compared to other rice varieties. In addition to traditional white or common rice, black rice have properties like unique flavor, aroma, color (red, purple, black), glossiness, stickiness, smooth texture, chemical composition, nutrition, esthetic, waxyness (very low amylose content) and superior processing qualities, which are increasing in demand in India, Pakistan and Thailand in Asia, the Middle East, Europe and the United States [5, 6, 7, 8]. The demand for various types of specialty rice has been increasing in recent years, which are sold for as much as 50% more than traditional rice cultivars[4]. In addition to this, black rice has been used in various traditional medicines and recently many researchers have reported that they have several health benefits in various studies and thus, black rice is being considered as the new super food by US scientists. One serving of black rice, even though contains some calories, but offers a high amount of flavanoid phytonutrients, important fiber, mineral content such as iron and copper and it is a good source of plant based protein which are hard to get to plant based eaters who rely on grains and legumes for protein [9].

Black rice is antioxidant-rich, containing anthocyanin [9, 10] and reported [11] that a spoonful of black rice bran provides the same amount or more anthocyanins than a spoonful of blueberries.

All phytochemical compounds would accumulate in the pericarp and testa or bran of the rice kernel. Antioxidant activities of paddy varieties containing colour pigments such as red Thai, black rice, red brown and dark purple had been intensively studied by Muntana and Prasong [12] and Yodmanee *et al.* [13], and they reported that rice with non color pigments contained lower phenolic content and antioxidant activities. Many studies have reported that black rice contains rich of anthocyanin and other polyphenolic compounds more abundantly than white rice [14, 15].

Ragi or finger millet (Eleusine Coracana) is one of the common cereals grown in India and a traditional food of South Indians since 1800 BC [16]. Indians consume cereals as the main staple providing 70-80% of total energy intake, the consumption of millets is very low compared to rice [17]. Millet refers to a number of different species, all of which are small-grained, annual cereal grasses [18, 1]. A number of minor millets exist, including finger (or ragi), proso and foxtail but as these account for less than 1% of the grains produced for human consumption and they are less important in terms of world food production. The finger millet is rich source of phytochemicals, dietary fibers, and polyphenols and cannot be ignored for enhancing the nutritional and therapeutic attributes of the formulated foods [19, 20]. Regular consumption of finger millet is known to reduce the risk of diabetes mellitus [21] and gastro intestinal tract disorders [22] and these health beneficiary aspects of millet could be attributed to its polyphenol contents. Owing to several health-promoting impacts associated with cereals and millets, the present investigation was carried out to screen the type of phytochemicals that are present in black rice varieties, brown rice and finger millets in the current study.

Methodology

Procurement of raw materials

Two varieties (Chakhao poireiton and Kavuni) of Black rice grown in two different regions of India were selected for the study. Chakhao poireiton, cultivated in place Nachou of Bishnupur district, Manipur and procured from farmer and Kavuni rice, grown in Tamil Nadu was procured from Satvika Bio- Foods private limited. Brown rice and finger millets were procured from local markets in Hyderabad.

Preparation of aqueous / methanolic sample extracts

In order to compare the total phenolic, flavonoids, antioxidant activity soluble compounds in different polarity solvents, the cereal samples were macerated and extracted in 2 types of solvents i.e. distilled water and 95% methanol. The standard procedures and tests [23, 24] were used to determine the presence of alkaloids, tannins, terpenoids, flavonoids, reducing sugar, anthraquinones, and saponins.

Firstly 2 g of sample was subjected to extraction by cold maceration in 100 ml of acidified methanol with 1N HCl (85:15) for 24 hrs followed by centrifugation at 3000 rpm for 10 min and filtered through Whattman No. 1 filter paper to obtain clear extracts. The clear filtrate was collected and preserved at 4 °C until further use.

Preliminary phytochemical screening

The preliminary tests for carbohydrate, alkaloids, proteins, amino acids, flavanoids, fixed oils, terpenoids, cardiac glycosides, steroids, tannins, phlobatins, phenols and quinones were carried out as per the procedure given by Harbourne (1993) [25].

Test for carbohydrates

To 2.0 ml of sample, 2 drops of Molisch reagent was added and shaken vigorously. To this 2.0 ml of Conc. H_2SO_4 was added from the sides of the test tube. A reddish violet ring appeared at the junction of two layers immediately to indicate the presence of carbohydrates.

Test for alkaloids

(a) Mayer's test: To a fraction of the extract, 1% HCl and 6 drops of Mayer's reagent (1.36 g of Mercuric chloride and 5.0 g of Potassium iodide in 100.0 ml of water) was added. An organic precipitate indicated the presence of alkaloids in the sample.

(b) Wagner's test: A fraction of extract was treated with Wagner's reagent (1.27 g of iodine and 2.0 g of potassium iodide in 100.0 ml water) and observed for the formation of cream colored precipitate.

(c) Hager's test: A few ml of extract was treated with Hager's reagent (saturated aqueous solutions of picric acid) and observed for the formation of prominent yellow colored precipitate.

Test for proteins: To 2.0 ml of sample extract, 1.0 ml of 40% NaOH and 1 to 2 drops of 1% $CuSO_4$ solution were added. A violet color indicated the presence of peptide linkage of the molecule.

Test for amino acids: To 2.0 ml of sample 2.0 ml of ninhydrin reagent was added and kept in water bath for 20 minutes at 60 °C. The appearance of purple color indicated the presence of amino acids in the sample.

Test for flavanoids: Each portion of sample was added with 5.0 ml ammonia followed by addition of few drops of conc. H_2SO_4. The development of a yellow color confirmed the presence of flavanoids and it disappeared on standing.

Test for fixed oils and fats: A few drops of 0.5 N alcoholic potassium hydroxide was added to a small quantity of extract along with a drop of phenolphthalein indicator. The mixture was heated on a water bath at 60 °C for 1 - 2 hrs. Formation of soap or partial neutralization of alkali indicated the presence of fixed oils and fats.

Test for terpenoids: To 5.0 ml of each extract, 2.0 ml of chloroform and 3.0 ml of conc. H_2SO_4 were added to form a monolayer of reddish brown coloration at the interface. This confirms a positive result for presence of terpenoids.

Test for cardiac glycosides: About 5.0 ml of each extract was treated with 2.0 ml of glacial acetic acid containing 1 drop of ferric chloride solution and was underplayed with 1.0 ml of conc. H_2SO_4. A brown ring at the interface indicated the deoxy-sugar characteristic of cardenolides. A violet ring might appear below the brown ring in the acetic acid layer where as a greenish ring might form just gradually throughout thin layer.

Test for steroids (Liebermann - Burchard test): Acetic anhydride (2.0 ml) was added to 0.5 ml of each extracts along with 2.0 ml of conc. H_2SO_4. The color change from violet to blue or green indicated the presence of steroids.

Test for saponins: Each extract was added with 20.0 ml of distilled water and was agitated in a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam indicated the presence of saponins.

Test for tannins: To 5.0 ml of extract few drops of 1% lead acetate was added and the formation of yellow precipitate indicated the presence of tannins.

Test for phlobatinins: Each extract was boiled with 1% HCl and the deposition of red precipitate indicated the presence of phlobatinins.

Test For Phenols

- **Ferric chloride Test:** A fraction of each of the extracts was treated with 5% Ferric chloride and observed for the formation of deep blue or black colour for the presence of phenols.
- **Liebermann's Test:** The extracts were heated with sodium nitrite and conc. H2SO4 solution diluted in water, cooled and added with excess of dilute NaOH. The formation of deep red or green or blue colour indicated the presence of phenols.

Test For Quinones: A small amount of each extract was treated with concentrated HCl and observed for the formation of yellow colour precipitate. The absence of yellow colored precipitate indicated that extracts have not undergone oxidation

Results and Discussion

The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently helps in drug discovery and development. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds [26].

Phyto chemicals also known as phyto nutrients are naturally occurring substances found in plants. These substances have been found to be beneficial to human health as well as possessing antioxidant activity [27]. Plant derived compounds are well known for their therapeutic values since ancient times. Phytochemicals could act as an antioxidant and anti-inflammatory substances. They play a vital role in detoxification of harmful and deleterious chemicals of the body. The phytochemicals, determined from different cereals in this study, are showed in table 1.

The current study showed that almost all the phytochemicals are strongly detected in Kavuni rice of both extracts compared to Chakhao Poireiton rice, Brown Rice and finger millet.

The presence of different phytoconstituents in kavuri rice of two different extracts may be responsible for several health benefits and therapeutic properties studied in various studies, and thus black rice varieties is being considered as the new super food by US scientists. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. The secondary metabolites (phytochemicals) and other chemical constituents of medicinal plants account for their medicinal value. For example, saponins have hypotensive and cardio depressant properties [28]. Glycosides are naturally cardio active drugs used in the treatment of congestive heart failure and cardiac arrhythmia [29]. The presence of saponins and glycosides in all the extracts might play a role in the cardio protective potential.

Proteins have identified in all the samples in both extracts except for brown rice whereas amino acids were not found in both extracts of all the samples. Phenols and cardiac glycosides were presented in both extracts of two rice samples. Flavonoids were presented in all the samples of both extracts but not identified in Chakhao Poireiton rice. Quinones were found in methanol extracts of Chakhao Poireiton rice and finger millet only whereas not identified in brown rice of both extracts. Phlobatinins were identified only in kavuri and Chakhao Poireiton rice of both extracts. Tanins and saponins were not found in all the samples except methanol extraction of Kavuri rice samples.

From this analysis it was observed that, methanol extract of all the samples detected more phytochemicals compared to aqueous solution. The ethanol extracts were generally more potent and less polar than the aqueous extracts probably because the active principles in plant dissolved more readily in and were better extracted. This is in agreement with many literatures reporting of differences in the activities of extracts obtained from the same morphological part of a plant using different solvents.

Conclusion

The study concluded that black rice varieties have many bioactive compounds that may help to regain immune power and maintain various metabolic reactions inside the body to dominate over a wide range of stress generated due to free radicals. Results obtained from the above study clearly indicate the presence of various types of phytochemicals in black rice extracts. The presence of huge amount of nutrient components in black rice varieties is helpful for the production of various nutraceuticals and antioxidant properties than the non colored rice varieties. Hence crude extract of colored rice bran / rice might act as a potential natural antioxidant source. The grains have greater scope in development and formulations of various value added and pharmaceutical products.

Table 1: Screening of phytochemicals in different cereals using methanol and aqueous solutions

Phytochemicals	Chemical Tests	Kavuni Rice		Chakhao Poireiton rice		Brown Rice (unpolished rice)		Finger Millet	
		Methanol	Distilled	Methanol	Distilled	Methanol	Distilled	Methanol	Distilled
Carbohydrates	Molish reagent test	+	+	+	-	+	+	+	-
Alkaloids	Mayers test	+	+	-	-	+	-	-	-
	Wagners test	+	+	-	-	+	-	-	+
	Hagers test	+	+	-	+	+	+	+	+
Proteins	Kjeldhal method	+	+	-	+	-	-	+	+
Amino acids	Ninhydrin reagent test	+	+	-	-	-	-	-	-
Test for fixed oils and fats	Foam test	+	+	-	-	-	-	-	
Terpenoids	Chloroform test	+	+	+	-	+	-	+	-
Cardiac glycosides	Glacial acetic acid test	+	+	+	-	-	-	-	-
Steroids	libermann-Buchard test	+	+	-	-	-	-	-	-
Saponins	Foam test	+	-	-	-	-	-	-	-
Tannins	$FeCl_{2\ test}$	+	+	-	-	-	-	-	-
Phlobatinins	HCL	+	+	+	+	-	-	-	-

Phenols	Ferric chloride test	+	+	+	+	-	-	-	-
	Liebarmanns test	+	+	+	+	-	-	-	-
Quinones	With conc HCL	-	+	+	-	-	-	+	-
Flavonoids	With ammonia solution	+	+	-	-	+	-	+	+

- = absence of compounds
Note: values are mean of three determinants
+ = presence of compounds indicates

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