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Original Research Paper

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PHYTOCHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITIES OF INDIAN CULTIVAR OF BLACK RICE (ORYZA SATIVA.L)

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ABSTRACT Black rice is a type of rice species Oryza sativa L. and is getting more attention recently because of its myriad health benefits. Black rice contains essential amino acids like tryptophan, lysine, vitamins such as vitamin B1, B2, folic acid and minerals such as iron, zinc, calcium, phosphorus and selenium. The present study was carried out to evaluate the phytochemical analysis, invitro antioxidant activity of the aqueous and ethanol extract of black rice. The total phenols and flavonoids were determined by standard methods and the antioxidant activity against hydroxyl radical, 1,1-diphenyl, 2- picryl hydrazyl (DPPH), superoxide and reduction potential were carried out. The results were analysed statistically which indicated the increase in anti oxidant activities with increasing concentration and ethanol extract showed more activity compared to the aqueous extract.

KEYWORDS : Oryza sativa; phenols; flavonoids; anti oxidants; phytochemicals.

INTRODUCTION

Oxidative stress is the major driving factor responsible for the initiation and progression of cancer, diabetes mellitus, cardiovascular diseases, neurodegenerative diseases, and inflammatory diseases among other syndromes (1). Free oxygen and nitrogen species are unstable molecules that are present in the environment (exogenous) and are also generated in the body (endogenous) during the normal aerobic metabolic processes in the body (2). Exogenous sources of free radicals include cigarette smoke, exposure to ozone, ionizing radiation such as X-rays, and drugs among others. On the other hand, endogenous sources of free radicals include the electron transfer chain reactions in the mitochondria, xanthine oxidase pathway, during disease states such as inflammation, ischemia, and reperfusion injury [3]. Evidence-Based Complementary and Alternative Medicine pathways, which in the normal physiologic state, maintain a steady equilibrium between prooxidants and antioxidants, thereby ensuring well-being (4). The search for better alternatives to synthetic antioxidants has triggered a significant research interest on dietary and medicinal plants that can inhibit, reverse, or ameliorate diseases caused by oxidative stress [5].

Body contains natural cleansing systems against free radicals and other reactive species. However, ROS production sometimes exceeds internal antioxidant capacity leading to oxidative stress. Therefore, external antioxidants have an important function in human health[6]. Certainly, a large number of synthetic drugs have been discovered in the past, but these drugs were found to have side effects. Therefore, researchers were focusing to develop new drugs from natural sources that are safe without having any side effects. One of the recent developments in the field of natural products is the exploration of a potent plant species. [8]. In this quest, the present study was carried out to evaluate the phytochemical analysis and invitro antioxidant activity of black rice. Black rice, also known as purple rice, is a range of rice types of the species Oryza sativa, some of which are glutinous rice. Black rice has a deep black colour and usually turns deep purple when cooked. Its dark purple colour is primarily due to its anthocyanin content, which is higher by weight than that of other coloured grains (9).

Experimental Details

Collection Of Plant Materials And Preparation Of Extracts

Oryza sativa plant seeds were collected and authenticated at the Department of Medicinal Botany, National Institute of Siddha, Chennai. (No. NISMB5152022). The seeds were powdered in a blender to obtain a coarse powder. 200g of each of the powdered Oryza sativa sample was extracted separately in distilled water and 99.9% ethanol using an orbital shaker. Then the solutions were filtered and the ethanol extracts were evaporated to dryness using a rotary evaporator while the aqueous extracts were initially frozen at -40°C and then dried using a freeze dryer. The different extracts were reconstituted in their respective solvents for use in the assays.

Phytochemical Screening

Total Phenolic Content

The content of phenols in the different extracts was determined spectrophotometrically by the Folin–Ciocalteu reagent according to the method of Ozkok et al.[10] A calibration curve was prepared with gallic acid as standard (0.025–0.125 mg/ml in 70% methanol v/v). To 0.5 ml of each of the gallic acid concentrations or extracts (mg/ml), 2.5 ml Folin–Ciocalteu reagent (previously prepared as 10% v/v dilution in distilled water) was added. Thereafter, 2 ml anhydrous sodium carbonate (7.5%) was added, producing a blue-colored solution. The mixtures were vortexed thoroughly and placed in a water bath for 30 min at 45°C. The absorbance was then read at 765 nm. The equation of the calibration curve obtained ($Y = 14.885x$; $R2 = 0.9961$) was used to establish the gallic acid equivalence (mg/ml). The total phenolic content was calculated using the formula: $T = C \times V/m$, where T is the total phenolic content, V is the volume of the extract (ml) used in the assay, C is the gallic acid equivalent (GAE) (mg/ml), and m is the weight of the pure plant extract used in the assay. Values were expressed as GAE per gram of dry plant extract (mg GAE/g). All assays were performed in triplicate.

Total Flavonoid Content

Flavonoid contents in the extracts were determined using the aluminum chloride method as described by Ozkok et al.[10] A calibration curve was prepared with quercetin (0.025–0.125 mg/ml in 80% methanol v/v). Briefly, 0.5 ml of the extracts (prepared at a concentration of 1 mg/ml) or the standard at the different concentrations was mixed with 3 ml of 95% ethanol, 0.2 ml of aluminum chloride (prepared as a 10% aqueous dilution), and 0.2 ml of 1 M potassium acetate, and the whole mixture was made up to 10 ml with distilled water. The resulting solutions, prepared in triplicate, were yellow and were thoroughly vortexed and allowed to stand for 30 min at room temperature, after which the absorbance was read at 420 nm. The equation of the calibration curve obtained ($Y = 11.922x$; $R2 = 0.9955$) was used to establish quercetin equivalence (mg/ml) and the total flavonoid content was calculated using the formula: $T = C \times V/m$, where T is the total flavonoid content, V is the volume of the extract (ml) used in the assay, C is the quercetin equivalent (mg/ml), and m is the weight (g) of the pure plant extract used in the assay. Values were expressed as quercetin equivalent per gram of dry plant extract (mg Qe/g).

Antioxidant Activity Assays

The antioxidant capacities of the different extracts were measured using DPPH radical scavenging activity, ferric-reducing power and nitric oxide (NO) scavenging activities.

1,1 Diphenyl-2-Picrylhydrazyl Radical Scavenging Activity

The method described by Liyana-Pathiranan and Shahidi[11] was used in this assay. 1 ml of the extracts or the standards at different concentrations (0.025–0.50 mg/ml), prepared in triplicates, was mixed with 1 ml of DPPH (0.135 mM) prepared in methanol. The mixtures were vortexed thoroughly and left in the dark for 30 min at room temperature.The absorbance was then measured spectrophotometrically at 517 nm. The percentage of DPPH scavenging activity of the extract or standard was calculated with the following formula: % DPPH radical scavenging activity = [(AC–AS)/AC] × 100, where AC is the absorbance of the control and AS is the absorbance of the test samples (extract or standard).

Ferric-Reducing Antioxidant Power

The method described by Aiyegoro and Okoh[12] was used for the determination of the ferric-reducing activities of the plant extracts. The assay is based on the reduction of ferric-tripyridyltriazine (Fe3+-TPTZ) complex by the action of electron-donating antioxidants at low pH to the ferrous form. Each extract or standard was initially prepared in distilled water in increasing concentrations from 0.025 to 0.5 mg/ml. 1 ml of each of the extract or the standard at the different concentrations was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide. The mixture was incubated for 20 min at 50°C. This was followed by the addition of 2.5 ml of TCA (10% w/v) and centrifugation at 3000 rpm for 10 min. Thereafter, 2.5 ml of the supernatant was withdrawn and mixed with 2.5 ml of distilled water and 0.5 ml FeCl3 (0.1% w/v). The absorbance was read at 700 nm with distilled water as blank. An increase in absorbance with increasing concentration of extract or standard corresponds to the formation of the bluish-green color of the reduced form of TPTZ. The average absorbance of the reactions performed in triplicate was obtained and plotted against the different concentrations of each extract and standard.

Nitric Oxide Scavenging Activity

The method described by Wintola and Afolayan[13] was used for the assay of the NO radical scavenging activity of the extracts. The procedure is based on the principle that sodium nitroprusside in aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ions. The nitrite ions are detected in solution by the Griess reagent which contains sulfanilamide and naphtylethylene diaminedihydrochloride. Compounds that scavenge NO compete with oxygen, leading to a reduced production of nitrite ions [14], For the essay, (0.5 ml of the extracts or standard was mixed with 2 ml of 10 mM sodium nitroprusside [prepared in 0.5 mM phosphate-buffered saline, pH 7.4]). The mixture was incubated for 2.5 h at 25°C. 0.5 ml of the mixture was mixed with 0.5 ml of Griess reagent (prepared by mixing 1 ml sulfanilic acid [0.33% in 20% glacial acetic acid] with 1 ml of naphthalene diaminedihydrochloride [0.1% w/v]). The mixture was incubated for 30 min at room temperature and the absorbance was measured at 540 nm. The percentage of NO scavenging ability of the plant extracts and standard compounds was calculated using the following formula: % NO scavenged = [(AC–AS)/AC] × 100, where AC is the absorbance of the control reaction and AS is the absorbance of the test samples (extract or standard).

Statistical analysis: All the data are expressed as mean ± SD from three observations for each concentration. A 'P' value of <0.05 and <0.01 was considered to indicate the statistical significance.

RESULTS AND DISCUSSION

Oryza sativa L. Indica is a well-known medicinal plant that is frequently prescribed in various indigenous systems of medicine especially those of Asia, Central America, Africa and Australia. Oryza sativa l. indica extracts, traditionally prepared (infusions, decoctions, tinctures of the leaves, flowers and seeds) by many widely separated cultures for cons of time for various uses have been shown by the application of modern scientific methods to indeed possess multiple disease ameliorating properties. Although there is enormous research on black rice cultivated in countries like Thailand, Japan, etc., not much research is carried out in the Indian cultivar.

An attempt has been made in the present work to study the phytochemicals and antioxidant activity of black rice grown in India. The results of the present findings are discussed as follows.

The yields of the extract obtained are O.sativa aqueous extract -15.4% and O.sativa ethanol extract – 7.8%.

Phytochemical Analysis of Oryza sativa l. indica

Phytochemicals are derived from plants and the term is often used to describe the number of secondary metabolic compounds found in plants. Phytochemical screening assay is a simple quick and inexpensive procedure that gives the researchers a quick answer to the various types of phytochemicals in a mixture and an important tool in analysis of bioactive compounds.

The phytochemical composition of the aqueous and ethanol extracts of O.sativa is presented in Table.1. The results indicate that the ethanol extract has high phenol and flavonoid content compared to the aqueous extract. This may indicate that ethanol may extract greater amounts of phytochemicals despite the relatively low yield from the original plant material compared to the aqueous extracts.

Assay Of Antioxidants

Dpph Radical Scavenging Activity

DPPH is a stable free radical that shows a maximum absorption at 570nm in methanol. When DPPH encounters proton donating substances such as an antioxidant and a radical species, the absorbance at 570nm disappears because the DPPH radical is scavenged. (15). On the basis of this principle, the radical scavenging effect of different concentrations (100μg/ml, 500μg/ml, and 1000μg/ml) of aqueous and ethanol extracts of Oryza sativa was measured, with ascorbic acid as the standard. The radical scavenging effect was found to increase with increase in concentration and the ethanol extract showed significantly greater radical scavenging activity compared with the aqueous extract. (Table 2)

Reduction Potential

The expression of antioxidant activity is thought to be concomitant with the development of reductones, as these species are known to be free radical chain terminators. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.[16] An increase in the absorbance of the extracts shows an increase in the reduction potential with increase in concentration (100μg/ml, 500μg/ml, and 1000μg/ml).

Nitric Oxide Scavenging Activity

Nitric oxide radical is a highly reactive species but it has a very low diffusion capacity. Nitric oxide radical is involved in lipid peroxidation which affects membrane fluidity, enzymes and receptors activity leading to apoptosis. If nitric oxide is generated near nucleic acids it reacts with purine and pyrimidine bases and 2-deoxyribose, leading to mutations which play an important role in carcinogenesis, as well as in neurodegenerative and cardiovascular diseases. (17,18). The

radical scavenging effect of Oryza sativa was found to be increased with increase in the concentration (100μg/ml, 500μg/ml, and 1000μg/ml) of the extracts. (Table 4)

SUMMARY AND CONCLUSION

Our study reveals the presence of potential phytochemicals in the extracts of Oryza sativa. With respect to the solvents used for extraction, the ethanol extracts had higher contents of phytochemicals than the aqueous extracts, an observation which correlated positively with the higher antioxidant activities exhibited by the ethanol extracts. Further studies are currently ongoing to investigate the acclaimed roles of the plants used in this study in the treatment of diabetes mellitus.

Table 1: Phytochemical analysis of aqueous and ethanolic extracts of Oryza sativa

Phytochemical constituents	OSA	OSE
Total phenols (mg GAE/g)	25±0.75	62.5±0.38
Total flavonoids (mg Qe/g)	9.23±1.23	27±0.45

Values are expressed as mean±SD of three replicates. mg GAE/g: Milligram gallic acid equivalent per gram of extract; mg Qe/g: Milligram quercetin equivalent per gram of extract; OSA – Oryza sativa aqueous extract, OSE – Oryza sativa ethanol extract.

Table 2 : DPPH Radical Scavenging Assay

Concentration (μg/ml)	Ascorbic acid (% Inhibition)	OSA (% Inhibition)	OSE (% Inhibition)
100	23.68 ± 1.62	19.74 ± 1.43**	21.17 ± 1.36**
500	36.48 ± 3.13	33.64 ± 2.68**	34.32 ± 1.92**
1000	56.46 ± 3.70	51.45 ± 3.08**	54.25 ± 3.01**
Ic50 (μg/ml)	2	3.5	4

Values are expressed as mean±SD of three replicates. Superscript represents significant differences at $P<0.05$. ; OSA – Oryza sativa aqueous extract, OSE – Oryza sativa ethanol extract.

Table 3 : FRAP Assay

Concentration (μg/ml)	Rutin (Absorbance)	OSA (Absorbance)	OSE (Absorbance)
100	0.19 ± 0.01	0.10 ± 0.006	0.15 ± 0.007
500	0.24 ± 0.009	0.18 ± 0.006	0.20 ± 0.005
1000	0.30 ± 0.008	0.24 ± 0.008	0.27 ± 0.006
Ic50 (μg/ml)	0.34	0.13	0.29

Values are expressed as mean±SD of three replicates. OSA – Oryza sativa aqueous extract, OSE – Oryza sativa ethanol extract.

Table 4 : Nitric Oxide Radical Scavenging Activity

Concentration (μg/ml)	Ascorbic acid (% Inhibition)	OSA (% Inhibition)	OSE (% Inhibition)
100	32.74 ± 1.83	27.21 ± 1.64**	30.93 ± 1.13**
500	37.69 ± 1.85	35.51 ± 1.71*	36.43 ± 2.09**
1000	48.17 ± 2.79	42.40 ± 1.68**	47.69 ± 1.34**
Ic50 (μg/ml)	6	8.6	8

Values are expressed as mean±SD of three replicates. OSA – Oryza sativa aqueous extract, OSE – Oryza sativa ethanol extract.

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