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Research Article

PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF WHEAT GRAINS AND SEEDLINGS P. Ravikumar¹, G. Shalini¹ and M. Jeyam²*

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ABSTRACT

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The phytochemical profiles and antioxidant activity of methanolic extract of Wheat Grains (WG) and 3 days old wheat seedlings (3dWS) were evaluated in the present study. Phytochemical analysis was performed for alkaloids, flavonoids, phenols, saponins, steroids, terpenoids, proteins and carbohydrates in WG and 3dWS extracts. Antioxidant activity was evaluated using DPPH and ABTS radical scavenging activity, total phenolic content and reducing power ability. In this study, the results of WG were compared with 3dWS. The results of phytochemical analysis showed the presence of all the above phytochemicals in the both 3dWS and WG extracts. When compared with WG, the 3dWS possesses high nutritional contents and antioxidant activity. Hence, it is suggested to add wheat seedling in the daily diet to reduce many diseases caused by antioxidant deficiency.

Keywords: Phytochemical analysis, antioxidant activity, 3 days old wheat seedlings, wheat grains, antioxidant deficiency.

INTRODUCTION

South Asian countries which include India, Pakistan, Nepal and Bangladesh are mostly having wheat as a stable food along with rice¹. In the world, India is the second biggest country in the wheat production² and has second place in wheat consumption after China³. Three types of wheat species are mostly cultivated in India. They are Triticum aestivum, Triticum durum and Triticum dicoccum⁴. Uttar Pradesh, Punjab, Harvana, Rajasthan, Madhya Pradesh, Gujarat and Bihar are the major states of India to produce large amounts of wheat. In the past four decades, approximately 312 wheat varieties were released by India in their 6 wheat zones⁵ and chapatti is the most eatable wheat product in India⁴. Wheat germ oil is highly contributed in the production of food products, biological insect control agents, pharmaceuticals and cosmetic formulations⁶ and is used to reduce plasma cholesterol, to decrease cholesterol absorption and to suppress platelet aggregation⁷. Wheat is highly nutritious crop which can be stored and transported easily. Protein, minerals, B-group vitamins and dietary fiber are rich in wheat^{8,9} and nutritional compositions of wheat grains are influenced by environmental conditions. Wheat play a major role in the production of animal feed, ethanol, beer, wheat based raw material for cosmetics and wheat straw composites. Researchers found that dietary fibers are high in wheat germ and bran which are used to treat many digestive disorders⁹. Wheat bran has hemicelluloses, protein, cellulose and high concentration of micronutrients such as 41-60 % of non starch polysaccharides (26 % are arabinoxylans), 15-20 % of protein and 10-20 % of residual starch¹⁰. Phytochemicals which include phenolic acids, flavonoids and carotenoids are present in the wheat bran and is used to keep health in good condition¹¹. Dieticians found that phytochemicals are essential sources of exogenous antioxidants¹². Epidemiological studies revealed that whole grains and whole grain products are used to reduce the risk of many chronic diseases such as cardiovascular disease, diabetes and cancer¹³⁻¹⁸. A combination of nutrition and pharmaceuticals are referred to as the word nutraceuticals and it specifies a component of food which mainly provide good

health and to prevent diseases¹⁹. Phytochemicals from whole grains are having antioxidant activity and helpful to scavenge free radicals²⁰. Antioxidant activity was found in six diverse varieties of whole wheat²¹. The present study is mainly focused to evaluate phytochemical profiles, nutritional values and antioxidant activity of methanolic extract of WG and 3 days old wheat seedlings (3dWS).

MATERIALS AND METHODS

Collection and preparation of plant material

Wheat (*Triticum aestivum*) seeds (Variety: HD2833 - Pusa Tripti) were obtained from Indian Agricultural Research Institute (IARI), Wellington, The Nilgiris, Tamil Nadu, India. Five hundred grams of seeds were washed and soaked in distilled water for 12 h and kept for germination up to 3 days. The germinated wheat seedlings were shade dried and homogenized to fine powder which was used for further processing. Another, five hundred grams of seeds were washed, shade dried, powdered and kept in airtight chamber.

Preparation of extracts

Three days old wheat seedlings powder (250 g) and wheat grain powder (250 g) were extracted with methanol (AR) using a Soxhlet apparatus for 48 h, filtered and the extracts were concentrated to 2 % of the original volume using Rotavapor, (Buchi-R210, Switzerland).

Qualitative phytochemical analysis

Qualitative phytochemical analyses of methanolic extracts 3dWS and wheat grain were determined by the following methods: Alkaloids (2 mL filtrate + 1 % HCL + steam, 1 mL filtrate + 6 drops of Mayer's reagent. Creamish precipitate indicates the presence of alkaloids), Flavonoids (2 mL of the filtrate + conc., HCl + magnesium ribbon. Pink- tomato red color indicates the presence of flavonoids), Saponins (0.5 mL filtrate + 5 mL distilled water. Frothing persistence indicates the presence of saponins), Steroids and Terpenoids (Liebermann-Burchard reaction: 2 mL of chloroform filtrate + 2 mL acetic anhydride + conc., H₂SO₄. Blue ring indicated the presence of terpenoids)²². Phenol (Test sample + sodium

hydroxide solution; yellow to red precipitate with in short time indicates the presence of phenolic compounds)²³. Proteins (2 mL extract + 1 mL ninhydrin solution. Purple color indicates the presence of protein), Carbohydrates (2 mL filtrate + few drops of α -naphthol solution + conc., H₂SO₄. Violet ring indicates the presence of Carbohydrates)²⁴.

Estimation of nutritional contents

Nutritional contents of methanolic extracts of 3dWS and wheat grains were estimated according to the method described by Lowry *et al*²⁵, Roe *et al*²⁶ and Folch *et al*²⁷ for protein, carbohydrates and lipids respectively. Thirty milligram of each sample was taken to estimate nutritional value.

DPPH radical scavenging activity

The antioxidant activity of methanolic extracts of 3dWS and wheat grains were determined using DPPH radical scavenging assay²⁸. Five different concentrations (20, 40, 60, 80 and 100 μ L) of the samples (30 mg/mL concentration) were made up to 0.1 mL with methanol to which 3.9 mL (0.025 g/ L) of the DPPH solution was added. A control solution was prepared without the addition of the extract. The absorbance was measured at 515 nm. The analysis was performed in triplicate and the scavenging activities of the extracts were calculated using the following equation:

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% scavenging Activity = [(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100 \%
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Where, Abs _{control} = absorbance of DPPH + methanol; Abs _{sample}= absorbance of DPPH + 3dWS / WG extracts

Total phenolic content

The total phenolic content was determined according to the method described by Siddhuraju and Becker²⁹. In the test tubes, 40 μ l of the extracts (30 mg/mL concentration) were taken and made up to volume of 1 mL with distilled water. Then 0.5 mL of Folin-Ciocalteu phenol reagent (1:1 with water) and 2.5 mL of sodium carbonate solution (20 %) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 minutes and the absorbance was recorded at 725 nm. The analysis was performed in triplicate and the results were expressed as gallic acid equivalents (GAE) using the following linear equation based on the calibration curve: y = mx + c (y = absorbance, x = phenolic content, and c = intercept).

$$Y = 0.045x - 0.033, R^2 = 0.997$$

Reducing power ability

The reducing power of the extracts was determined according to the method of Yildrim *et al.*³⁰ In the test tubes, 40 µl of the extracts (30 mg/mL concentrations) were taken and made up to 1 mL with methanol, further mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [K₃Fe(CN)₆] (2.5 mL, 1 %). After incubating the mixture at 50°C for 20 minutes, 2.5 mL of trichloroacetic acid (10 %) was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 mL, 0.1 %). The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. A control solution was prepared without the addition of the extract. The analysis was performed in triplicate and the results were expressed in ascorbic acid equivalents (AAE) using the following linear equation based on the calibration curve:

y = mx + c (y = absorbance, x = reducing power and c = intercept) Y = 0.004x + 0.029, R² = 0.999

ABTS radical scavenging assay

The total antioxidant activity of the extracts was measured by ABTS radical cation decolourization assay according to the method of Re et al³¹ described by Siddhuraju and Manian³². ABTS radical was produced by reacting 7 mM ABTS aqueous solution with 2.4 mM potassium per sulfate in the dark for 12-16 h at room temperature. Prior to assay, this solution was diluted in methanol and equilibrated at 30°C to give an absorbance at 734 nm of 0.700 ± 0.02 and in the present study, the stock solution was obtained in the ratio of 1:53 v/v. One mL of ABTS stock solution was added to five different concentrations (2, 4, 6, 8 and 10 µL) of test samples (10 mg/mL concentration). The control sample was prepared without the test sample solution. After the initial mixing, all the tubes were kept at 30°C exactly for 30 minutes and absorbance was measured at 734 nm. The analysis was done in triplicate and the percentage scavenging activities of the extracts were calculated using the following equation:

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ABTS radical scavenging activity (%) = [(Abs _{control} - Abs _{sample}) /Abs _{control}] \times 100 %
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Where, Abs _{control} = absorbance of ABTS solution + methanol; Abs _{sample} = absorbance of ABTS solution + 3dWS/wheat grains extracts

RESULTS

Preliminary phytochemical analysis

Preliminary phytochemical analysis was performed for the methanolic extracts of 3dWS and WG and the results were tabulated (Table 1). All the groups of phytochemicals tested in this study were present in both the samples.

Estimation of nutritional contents

Nutritional contents of methanolic extracts of 3dWS and WG were estimated (Table 2). The results showed that protein was significantly increased in 3dWS and increase of lipids was insignificant in the seedlings. The carbohydrate content of 3dWS decreased to a significant level.

DPPH radical scavenging activity

DPPH radical scavenging activity of methanol extracts of WG and 3dWS were evaluated and the results are in Figure 1 and 2, respectively. The radical scavenging activity increased two fold in the seedlings.

Total phenolic content and Reducing power ability

Total phenolic content and reducing power ability of methanol extracts of 3dWS and WG were evaluated and results were tabulated (Table 3).

ABTS radical scavenging assay

ABTS radical scavenging activity of methanol extracts of WG and 3dWS were tested and the results are shown in Figure 3 and 4, respectively which showed significant increase in the seedlings.

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S. No.	Name of the test	3 days old wheat seedlings	Wheat grains
1	Alkaloids	+	+
2	Flavonoids	+	+
3	Phenols	+	+
4	Saponins	+	+
5	Steroids	+	+
6	Terpenoids	+	+
7	Protein	+	+
8	Carbohydrate	+	+

Table 1: Phytochemical Analysis of Methanolic Extracts of 3d Wheat Seedlings and Wheat Grains

(+) =presence; (-) =absence

Table 2: Nutritional Contents of Methanolic Extracts of 3dws and Wheat Grains

S. No.	Nutritional contents	3days old wheat seedlings (mg/g)	Wheat grains (mg/g)
1	Protein	326 ± 11.08	305.84 ± 10.80
2	Carbohydrate	36.79 ± 2.81	46.14 ± 3.78
3	Lipids	20.63 ± 1.50	19.78 ± 1.10

Table 3: Total Phenolic Content and Reducing Power Ability of Methanolic Extracts of 3dws and Wheat Grains

Test	3days old wheat seedlings	Wheat grains
Total phenolic contents*	3.11 ± 0.07	2.54 ± 0.04
Reducing power ability**	10.83 ± 0.42	2.5 ± 0.21

Note: *Results are expressed in mg Gallic Acid Equivalent (GAE) /g of extract; ** mg Ascorbic Acid, Equivalent (AAE) /g of extract

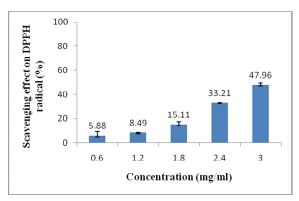


Figure 1: DPPH radical scavenging activity of methanol extract of wheat grains

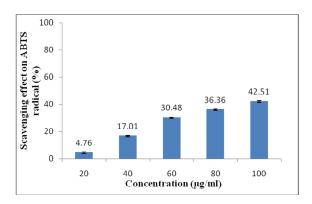


Figure 3: ABTS radical scavenging activity of methanol extract of wheat grains

DISCUSSION

Fruits, vegetables and whole grains have phytochemicals which are bioactive, non-nutrient, naturally occurring plant compounds³³ and phytochemicals determine the health benefits of whole grains²⁰. In the present study, most of the phytochemicals such as alkaloids, flavonoids, phenols, saponins, steroids, terpenoids, protein and carbohydrate were

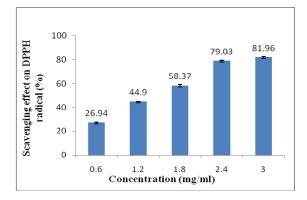


Figure 2: DPPH radical scavenging activity of methanol extract of 3d wheat seedlings

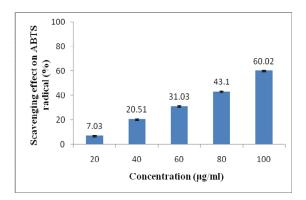


Figure 4: ABTS radical scavenging activity of methanol extract of 3d wheat seedlings

present in both 3dWS and WG. From the results of nutritional contents, the protein content of 3dWS was higher than the WG. When carbohydrate content of 3 days old wheat seedlings was compared with wheat grains, it was low in 3dWS but lipid content was slightly high in 3dWS. Antinutritional compounds like phytic acid, phytate and polyphenols are reduced while grain is soaking and it also increases health benefits³⁴. In the present study, 3dWS were also prepared after soaking wheat grains which may decrease the anti-nutritional compounds and may increase the nutritional value. Fardet $et al^{35}$ reported that whole grains are also useful to reduce the risk of age related diseases such as diabetes, cardiovascular diseases and cancer. Lv et al³⁶ states that nutrient compositions are influenced by genotype (G), growing environment (E) or interaction between genotype and growing environment. The present study is also useful to know the nutritional composition of Indian wheat variety Pusa Tripti (HD 2833). Ragaee et al³⁷ reported DPPH and ABTS radical scavenging activity and total phenolic content of wheat flour and whole grain cereals and the results when compared with the present study showed that 3dWS had high antioxidant activity. Sprouting grains are having many health benefits. Sprouting increased calcium, vitamins and enzymatic activity that further aids in easy digestion. Sprouting of wheat grains is also useful to reduce anti nutrients (like poly phenols, phytate, tannins etc.). Due to low glycemic index of wheat seedlings, it gives good heart health and aids to diabetic patients³⁸. Hence, the present study concludes that the nutritional contents and antioxidant activity of three days old wheat seedlings is higher than wheat grains. Further, it is suggested that daily consumption of wheat seedlings in the diet is helpful to reduce the risk of chronic diseases such as asthma, cancer, cardiovascular diseases, obesity and diabetes.

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