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Determination of Antioxidant Properties and the Bioactive Compounds in Wheat (*Triticum aestivum* L.)

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Abstract

The antioxidant property and bioactive compounds' activity of two wheat varieties have been investigated in this research. To conduct this experiment, leaves and seeds of wheat were extracted by methanol and ethanol. For DPPH free radical scavenging assay, plant extracts showed 50 % scavenging ability compared with standard antioxidant (BHT). The results revealed that the Kheri leaf extracts had 2.17, 5.03, 11.74, 25.44, 39.64, 49.41 and 54.34% with various doses which clearly shows that it is capable of scavenging the 50 % DPPH. The Kheri seed extracts showed IC_{50} value of 55µg/mL for scavenging the 50 % DPPH. On the other hand, the Pavon 76 leaf and seed extract showed an IC_{50} value of 80 µg/mL and 90µg/mL at different concentrations. The ethanol extracts of the two tested genotypes had an abstemiously-high antioxidant effect though this effect was not approximate to the synthetic antioxidant (BHT). Moreover, the extracts of various solvents were tested to identify the nature of phytochemical constituents which exposed several bioactive compounds such as alkaloid, flavonoids, phenols, saponins, amino acids and carbohydrates present in trace amount in the case of whole-plant extracts. The antioxidant property in whole grains may be responsible for the health benefits of the whole-grain consumption of wheat.

Keywords: Antioxidant activity, Bioactive compounds, DPPH assay, Phytochemical analysis, *Triticum aestivum.*

1. Introduction

Secondary metabolic compounds found in plants are effective sources of protection against insect attacks, plant diseases and chronic microbial effects. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, cardiac glycosides, steroids and saponins (Nithya *et al.,* 2011; Morshed and Islam, 2015). The edible oils and fats are generally oxidized in the presence of oxygen and light resulting in the formation of reactive oxygen species which have been associated with cancer, cardiovascular diseases, inflation and aging (Siddhuraju and Beeker, 2003). The consumption of oxidized lipids is associated with oxidation of biological membranes, genotoxicity and tocopherol inhibition (Sikwese and Duodu, 2007). Antioxidants encounter the reactive oxygen species, and reduce the risk associated with lipid oxidation. There has been an increasing interest on the part of the food industries and preventive medicines to replace synthetic antioxidants with those of safer and more natural origins as stated by Shahidi (1997) and Chatha *et al.* (2006). Cereals and other crops antioxidants have gained substantial interest over the last few years (Bhattacharjee *et al.,* 2015; Paul *et. al.,* 2017). Fleischman *et al.* (2016) evaluated antioxidant capacity to incorporate wheat bran into an extruded snack. They reported that extrudates made with the addition of red (37.5 %) and purple (37.5 %) bran had higher values compared to the other treatments. They also mentioned that the control, red, and white bran treatments had less antioxidant activity after extrusion compared to purple bran supplemented extrudates. Purple and red brans may serve as viable functional ingredients in extruded foods given their higher antioxidant activities. Giordano *et al.* (2017) studied the chemical composition of pigmented wheat namely yellow, purple and blue types and the distribution of the bioactive compounds in their roller-milled and pearled fractions and were compared with conventional wheat varieties that are of the red and white types. Roller-milling promoted the recovery of total dietary fiber, β-glucans, phenolic acids and anthocyanins in the bran fraction, which resulted also in a higher total antioxidant activity than the refined flour. There is a demand to find effective natural antioxidants to replace synthetic ones, which has also added to the amount of research done on cereal antioxidants such as tocopherols, tocotrienols, and phenolic acids. Tocopherols as well as other bioactive components (phytosterols,

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folates, lignans, and alkenresorcinols) have been shown to be located on the outer parts of the cereal kernel, especially the bran (Nystrom *et al.,* 2005; Mariod *et al.,* 2010). Natural antioxidants are constituents of many fruits and vegetables, and they have attracted a great deal of public and scientific attention because of their anti-carcinogenic potential and other health-promoting effects. However, consumers are quite cautious about the quality of their diet and its chemical additives to replace synthetic antioxidants with those of safer and more natural origins. This has prompted the investigation and characterization of active natural antioxidant compounds in various plant-derived foods (Zuo *et al.,* 2002). Free radical induces oxidative damage to biomolecules that cause cancer, aging, neurodegenerative diseases, atherosclerosis, malaria and several other pathological events in living organisms. Antioxidants which scavenge free radicals are known to possess an important role in preventing these free radical induced diseases. There is an increasing interest in the antioxidants effects of compounds derived from plants, which could be relevant to their nutritional incidence and their role in health. In this study, the antioxidant activity test is undertaken with determination of total antioxidant capacity (TAC) and DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging assay.

2. Materials and Methods

As plant materials, mature leaves and seeds of the wheat varieties Kheri and Pavon 76 were collected from the field grown plants of the Institute of Biological Sciences, University of Rajshahi, Bangladesh. DPPH (Sigma Aldrich, India), butylated hydroxyl toluene (BHT) as standard antioxidants, sulphuric acid, ammonium molybdate, sodium phosphate, and ascorbic acid from E-Merck, Germany were used in the investigation.

2.1. Preparation of Extracts, DPPH and BHT Solution

Leaves and seeds of the plants were collected from the culture field which belongs to the Institute of Biological Sciences at the University of Rajshahi, Bangladesh in January of 2016. They were then sliced and chopped into small pieces, dried under shade, and were powdered by a hand grinder. They were weighed and placed in separate conical flasks to add solvents. Methanol and ethanol were used (200 g × 600 mL × 2 times) successively each of which took forty-eight hours on a shaker. Each of the extract filtration was done by Whatman filter paper (made in USA) at a twenty-four-hour interval in the same flask followed by evaporation until the extract was left as a scum. The extracts were then removed to glass vials, and were preserved in a refrigerator at 4°C with proper labeling. One milligram of each plant sample extracts was taken in a vial and added carefully 1 mL of solvent (methanol or ethanol) and dissolved by inverting each solution was containing 1 µg extract/1 µL. Four milligram DPPH was taken in a test tube carefully added 100 mL of solvent (methanol) and mixed then mixed gently and kept it in dark condition until uses. Then BHT 1 mg taken in a test tube in addition with 1 mL of solvent (methanol) and shacked it for dissolving. As standard antioxidant BHT was used to determine the scavenging ability which was compared with 50% DPPH and this was the IC_{50} value of the BHT for scavenging DPPH concentration.

2.2. DPPH Radical Scavenging and Measurement of the Absorbance of Solution

The DPPH scavenging activity was tested by using different concentrations of BHT and a control for comparison. Then the BHT stock solution was taken for 5, 15, 30, 60, 100, 200 and 400 µL solution and 995, 985, 970, 940, 900, 800 and 600 µL solvent (methanol or ethanol) were added in each concentration, respectively. Therefore, the concentrations of the BHT in the first seven samples were taken 5, 15, 30, 60, 100, 200 and 400 µg/µL, respectively. Finally, 3 mL of the DPPH solution were added to each of the samples to make a final volume of 4 mL for every sample. The samples were then incubated at room temperature for thirty minutes in the dark to complete the reaction. After thirty minutes of incubation absorbance of the solutions were measured at 519 nm using a spectrophotometer against blank. All the measurements were repeated three times for accuracy of the experiment, and the mean value of the absorbance was used for further calculations. A typical blank solution contained all reagents except BHT.

2.3. DPPH Scavenging Activity and IC50 Value of BHT

The percentage (%) inhibition activity was calculated from the following equation: $I = \frac{A_0 - A_1}{A_0} \times 100$; where, I is the percentage of inhibition, A_0 is the absorbance of the control and A_1 is the absorbance of the BHT.

2.4. DPPH Free Radical Scavenging Assay of Extract

For the DPPH scavenging activity test, different concentrations namely 5, 15, 30, 60, 100, 200, and 400 µg/µL solution were taken from stock solution of Kheri and Pavon 76 seed and leaf extracts of ethanol and methanol but without adding extract solution in control. Then 995, 985, 970 and 940, 900, 800, 600 µL ethanol and methanol solvents were added. Therefore, the concentration of the extract in test tubes was 5, 15, 30, 60, 100, 200, and 400 µg/µL as previously mentioned. Finally, 3 mL of DPPH solution added to each of the test tube. As a result, total volume of each of test tube was 4 mL. The test tubes were then incubated at RT (room temperature) for 30 minutes in dark to complete the reaction.

2.5. DPPH Scavenging Activity and IC50 Value of the Extract

The measurement of the absorbance, calculation of the percentage of the DPPH scavenging activity and the IC_{50} value of the extract were almost similar to the experimental procedure of BHT. Although, here, a blank solution was used and contained all reagents except the extract during the measurement of absorbance using a spectrophotometer.

2.6. Total Antioxidant Capacity Test

The total antioxidant capacity (TAC) of the samples/standard was determined by the methanol and ethanol with some modifications. Six autoclaved test tubes were needed to perform the total antioxidant capacity (TAC) test of the targeted plant extract at different concentrations. The test tubes were labeled mentioning the

concentration 1, 2, 3, 4, 5 and control. The five test tubes were respectively filled with 5 µg, 10 µg, 20 µg, 50 µg, and 80 µg/mL from the stock solution of the Kheri and Pavon 76 leaf and seed extracts. The stock solution was not added to the control test tube. The samples were mixed with 3 ml of reaction mixture containing 0.8 M sulphuric acid, 14 mM sodium phosphate and 0.4 % ammonium molybdate which were added to the test tubes. The test tubes were incubated at 95°C for ten minutes to complete the reaction. The absorbance was measured at 695 nm using a spectrophotometer against blank after being cooled at room temperature. Here, ascorbic acid was used as standard. A typical blank solution containing 3 mL of the reaction mixture, and the appropriate volume of the same solvent used for the samples/standard were incubated at 95°C for ten minutes and the absorbance was measured at 695 nm. The increased absorbance of the reaction mixture indicated an increase in the total antioxidant capacity.

2.7. Phytochemical Analysis

Phytochemical tests were carried out using various solvents while methanol, ethanol and chloroform were subjected to routine qualitative chemical analysis to identify the nature of phytochemical constituents by the standard procedures (Nithya *et al.,* 2011) as mentioned below:

Alkaloids: 1 mL of the extract was added to 2-3 drops of Mayer's reagent (dissolve 1.36 g of mercuric chloride in 60 mL of H_2O and poured onto the solution of 5 g of potassium iodide in 100 mL of H_2O). Cream color or yellow precipitation indicates the presence of alkaloids.

Terpenoids (Salkowski test): 1 mL of the extract was mixed with 2 ml of chloroform and concentrated H_2SO_4 (3 mL) was carefully added to form a layer. A reddish-brown color of the interface indicates positive results for the presence of terpenoids.

Flavonoids: In this test, 1 mL of the extract was dissolved and added in diluted NaOH + HCl. A yellow colorless solution indicates the presence of flavonoids.

Tannins: 1 mL of the extract was added to few drops of 1 % lead acetate. Yellow precipitations indicate the presence of tannins.

Phlobatanins: 1 mL of the extract was filtered and dissolved in distilled water. The filtrate was boiled with 2 % HCl solution. Red precipitations indicate the presence of phlobatanins.

Coumarins: 3 mL of 10 % NaOH was added to 2 mL of aqueous extract. The formation of the yellow color indicates the presence of coumarins.

Steroids: 1 mL of the extract was dissolved in 10 mLof chloroform, and an equal volume of concentrated H_2SO_4 was added by sides of the test tube. The upper layer turned red and the H_2SO_4 layer showed yellow with green fluorescence. This indicates the presence of steroids.

Phenols: Phenols were tested by adding 2 mL of ferric chloride solution to 2 mL of the plant extract. Appearances of a blue green color solution indicate the presence of phenols.

Saponins: Saponins were tested by boiling 5 mL of the extracts in 10 mL of distilled water in a test tube and were shaken vigorously for about thirty seconds. The test tube was allowed to settle for half an hour. The formations of froth indicate the presence of saponins.

Amino acids: 1 mL of the extract was treated with few drops of Ninhydrin reagent. The appearance of the purple color indicates the presence of amino acids.

Glycosides: The extract and alpha napthol (one ml each) were added alongside chloroform. The development of color and the results were recorded. Developments of the violet color indicate the presence of glycosides.

Carbohydrates: An alcoholic solution of substance was added to 10 % aqueous solution of alpha-naphtali. After shaking, concentrated H_2SO_4 was added along the side of the tube. The violet ring at the junction of two liquids confirms the presence of carbohydrates.

3. Results and Discussion

An antioxidant tests on the varieties of Kheri and Pavon 76 are scanty, however there are some reports on other varieties of wheat. For this reason, much attention has been focused on these two wheat varieties. These varieties are also agronomically superior in Bangladesh. Methanol and ethanol extracts were used at different concentration levels and were compared with the standard antioxidant BHT. There are some reports that explored the antioxidant effect (DPPH free radical scavenging activity) of BHT standard at seven concentration levels 5, 15, 30, 60, 100, 200 and 400 µg/mL, and was found average 24.07, 30.25, 35.87, 63.97, 88.50, 95.83 and 98.57 percentage values with BHT IC_{50} value 50 µg/mL. That means BHT is capable of scavenging the 50 % DPPH at the concentration of 50 µg/mL. Table 1 showed that the IC_{50} of the wheat varieties of Kheri and Pavon 76 leaf and seed methanol and ethanol extracts were 180, 100, 100, 190 µg/mL and 205, 55, 80, 90 µg/mL, respectively. Hence the leaf and seed methanol extract showed a higher antioxidant effect than the methanol, but this effect was not approximate to the synthetic antioxidant (BHT). The percent DPPH free radical scavenging activity of the leaf and seed methanol extract ranged from 0.51 to 66.62 %, respectively in the above-mentioned doses. On the other hand, the DPPH free radical scavenging activity of the Kheri and Pavon 76 leaf and seed ethanol extract were 2.17, 5.03, 11.74, 25.44, 39.64, 49.41, 54.34; 3.16, 16.27, 35.60, 58.58, 70.41, 77.91, 80.18 and 4.93, 10.06, 17.65, 48.82, 60.06, 64.89, 70.81; 0.39, 1.38, 17.16, 33.13, 51.68, 71.79, 77.91 %, respectively at the same afore mentioned doses (Table 1). So, it is clear that the leaf extract of Kheri and Pavon 76 of both solvents is capable of scavenging the 50 % DPPH at the dose of 180, 205 and 100, 80 µg/mL. Similarly, the seed extracts showed IC_{50} as 100, 55 and 190, 90 g/mL at the same test at diverse concentration levels. It was observed that the Kheri and Pavon 76 ethanol extract had an abstemiously high antioxidant effect although this effect was not fairly accurate to the synthetic antioxidant (BHT) (Table 1).

Table 1. DPPH free radical scavenging activity using methanol and ethanol extracts of selected seven samples and BHT with various concentrations.

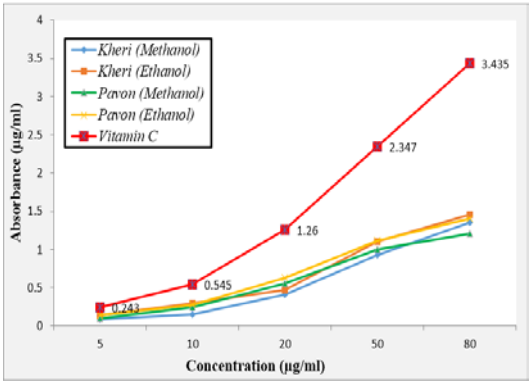
Samples		Conc. (µg/mL)	Solvents: Methanol Absorbance Mean ± SD	Methanol % of DPPH scavenging	Methanol IC_{50} (µg/mL)	Ethanol Absorbance Mean ± SD	Ethanol % of DPPH Scavenging	Ethanol IC_{50} (µg/mL)
Kheri	Leaf	5	0.688 ± 0.062	17.52	180	0.923 ± 0.11	2.17	205
		15	0.500 ± 0.308	16.88		0.896 ± 0.109	5.03	
		30	0.492 ± 0.303	18.41		0.832 ± 0.101	11.74	
		60	0.479 ± 0.295	20.84		0.703 ± 0.085	25.44	
		100	0.384 ± 0.240	38.11		0.569 ± 0.069	39.64	
		200	0.297 ± 0.183	54.60		0.477 ± 0.058	49.41	
		400	0.251 ± 0.156	62.92		0.430 ± 0.052	54.34	
	Seed	5	0.559 ± 0.032	30.18	100	0.873 ± 0.091	3.16	55
		15	0.554 ± 0.033	30.82		0.749 ± 0.089	16.27	
		30	0.503 ± 0.033	37.34		0.566 ± 0.075	35.60	
		60	0.44 ± 0.025	46.16		0.349 ± 0.066	58.58	
		100	0.417 ± 0.025	50.00		0.237 ± 0.065	70.41	
		200	0.392 ± 0.026	53.58		0.166 ± 0.066	77.91	
		400	0.340 ± 0.049	62.79		0.141 ± 0.072	80.18	
Pavon 76	Leaf	5	0.595 ± 0.205	11.25	100	0.866 ± 0.096	4.93	80
		15	0.529 ± 0.122	32.99		0.818 ± 0.091	10.06	
		30	0.473 ± 0.125	39.90		0.746 ± 0.084	17.65	
		60	0.433 ± 0.125	45.01		0.451 ± 0.059	48.82	
		100	0.389 ± 0.132	50.13		0.345 ± 0.053	60.06	
		200	0.35 ± 0.135	56.52		0.298 ± 0.051	64.89	
		400	0.287 ± 0.106	65.47		0.242 ± 0.050	70.81	
	Seed	5	0.748 ± 0.042	0.51	190	0.909 ± 0.101	0.39	90
		15	0.727 ± 0.040	3.11		0.900 ± 0.100	1.38	
		30	0.694 ± 0.039	7.68		0.756 ± 0.084	17.16	
		60	0.527 ± 0.029	29.68		0.610 ± 0.068	33.13	
		100	0.485 ± 0.026	35.30		0.441 ± 0.049	51.68	
		200	0.343 ± 0.018	54.11		0.257 ± 0.029	71.79	
		400	0.248 ± 0.013	66.62		0.202 ± 0.022	77.91	
					Control			
BHT	Conc. (µg/mL)		Absorbance Mean ± STD	% of DPPH scavenging	IC_{50} (µg/mL)			
	5		0.581 ± 0.007	24.07				
	15		0.392 ± 0.002	30.25				
	30		0.332 ± 0.002	35.87				
	60		0.281 ± 0.007	63.97	50			
	100		0.092 ± 0.002	88.50				
	200		0.356 ± 0.001	95.83				
	400		0.024 ± 0.006	98.57				

The total antioxidant effect of wheat (leaf and seed) of methanol and ethanol extracts among the five doses (5, 10, 20, 50 and 80 µg/mL) were evaluated and analyzed the absorbance of the total antioxidant activity of Kheri and Pavon 76 with the absorbance of standard vitamin C (µg/mL) (Table 2). Compared to the standard vitamin C, the methanolic and ethanolic extracts of the two varieties showed lower antioxidant activity at all concentrations. The increase in total antioxidant activity was observed with increasing the concentration of the extracts.

The results of the phytochemical activities showed that the bioactive compounds such as alkaloid, flavonoids, phenols, saponins, amino acids and carbohydrates were present in trace amount in the leaf extracts of wheat. Whereas, terpenoids, tanins, phloba tanins, steroids, phenols and glycosides, were not detected in any of the plant extracts (Table 3).

Table 2. Absorbance of wheat leaf and seed and vitamin C with various concentrations for total antioxidant activity assay.

Plant parts	Conc. (µg/ml)	Variety				Absorbance of vitamin C (µg/ml)
		Kheri		Pavon 76		
		Methanol	Ethanol	Methanol	Ethanol	
Leaf	5	0.12	0.205	0.19	0.261	0.243
	10	0.24	0.436	0.462	0.509	0.545
	20	0.62	0.712	0.874	0.900	1.26
	50	1.14	1.195	1.32	1.477	2.347
	80	1.46	1.436	1.47	1.806	3.435
Seed	5	0.091	0.136	0.098	0.133	0.243
	10	0.148	0.29	0.245	0.278	0.545
	20	0.41	0.471	0.557	0.629	1.26
	50	0.926	1.099	0.996	1.107	2.347
	80	1.346	1.454	1.2	1.403	3.435



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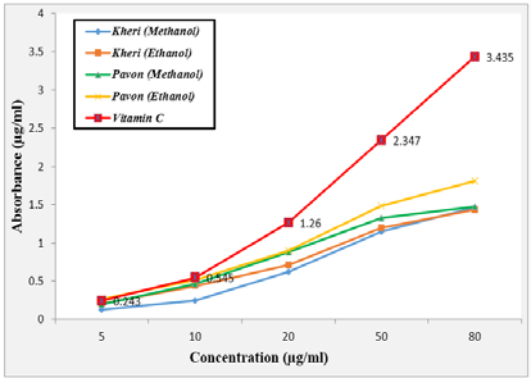

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Figure 2. Standard absorbance of vitamin C (µg/ml) of methanol for both leaf and seed (A) and ethanol only for seed (B) extract at different concentration levels.

Table 3. Phytochemical analysis with various extracts and methods of wheat

Name of Photochemical	Test methods	Observation (colure /precipitation)	Extracts	Remarks
Alkaloids	Mayer's test	Creamy color precipitate	Methanol	+
			Ethanol	+
Terpenoids	Salkowsky test	No change is observed	Methanol	-
			Ethanol	-
Flavonoids	Alkaline reagent test	A yellow solution that turns colorless	Methanol	+
			Ethanol	+
Tannins	Lead acetate test	No change is observed	Methanol	-
			Ethanol	-
Phlobatanins	General test	No red precipitate observed	Methanol	-
			Ethanol	-
Steroids	Libermann-Burchard's test	No change is observed	Methanol	-
			Ethanol	-
Phenols	Ferric chloride test	No change is observed	Methanol	+
			Ethanol	+
Saponins	Frothing test	Formation of froth	Methanol	+
			Ethanol	+
Amino acids	Ninhydrin test	Appearance of blue color	Methanol	+
			Ethanol	+
Glycosides	General test	No change is observed	Methanol	-
			Ethanol	-
Carbohydrates	Molisch Test	Violet ring at the junction of two liquids	Methanol	+
			Ethanol	+

NB: (+) = Presence of constituents and (–) = Absence of constituents.

Antioxidant activities (by ferric reducing ability of plasma assay) of steamed bread made from whole wheat flour, partially rebranded grain flour, and refined flour were 23.5 %, 21.1 %, and 31.6 % lower, respectively, than the corresponding values of flour which suggested that the black whole wheat flour and partially rebranded grain flour are beneficial to human health (Li *et al.*, 2015). Free radicals contribute to cancer, atherosclerosis, malaria, and rheumatoid arthritis and neurodegenerative diseases (Okezie, 1999). In the scientific and medical communities, antioxidants are considered to have the ability to scavenge free radicals and reduce oxidative damage (Yu *et al.*, 2002; Mellen *et al.*, 2008). However, food processing also affects the antioxidant properties of foods to different extents. Wu *et al.* (2004) found that processing methods (including cooking and hulling) affected the oxygen radical absorbance capacity. The antioxidant activity is high compared with the effect of synthetic antioxidant (BHT) (Table 1). The free radical DPPH antioxidant activity test of wheat varieties showed that the standard BHT is capable of the scavenging of DPPH 50 % at the concentration of 50 µg/ml. The experimental result indicates that the IC_{50} value of all wheat varieties of

methanol and ethanol extracts are greater than the standard BHT. The total antioxidant capacity of wheat (leaf and seed) is higher than the standard (BHT) at five different concentrations. The antioxidant capacity of the methanolic and ethanolic extract of the wheat sample is attributed to their chemical composition and phenolic content. The present findings revealed the antioxidant activity of wheat compared with the DPPH free radical scavenging activity of BHT. The average percentages of 24.07, 30.25, 35.87, 63.68, 88.50, 95.83 and 98.57 at different concentration levels with BHT IC_{50} value of 50 µg/mL were found. That means BHT is capable of scavenging 50 % DPPH at the concentration of 50 µg/mL. Results showed that the IC_{50} value of the wheat varieties of Kheri and Pavon 76 leaf and seed are 180, 100, 100 and 190 µg/mL respectively which supports the above-mentioned findings.

The results of the phytochemical screening of wheat revealed the presence of trace amounts of alkaloid, flavonoids, phenols, saponins, amino acids and carbohydrates in all the solvent extracts (methanol and ethanol). On the other hand, terpenoids, tanins, phlobatanins, steroids, phenols and glycosides, were not detected in any of the plant extracts. The presence of these secondary metabolites in wheat confirms their antibiotic properties and usefulness for the traditional medicine practitioners to treat various ailments. Flavonoids are also known to have a wide array of therapeutic activities such as antihypertension, antirheumatism, antimicrobial, diuretic and antioxidants (Burkill, 1988; Trease and Evans, 2002). Alkaloids are formed as metabolic byproducts and have been reported to be responsible for the antibacterial activity (Doughari, 2006; Bhattacharjee and Islam, 2015). The presence of saponin enhanced the antimicrobial activity against the pathogenic microorganisms. More lipophilic flavonoids may also disrupt microbial membranes (Tsuchiya *et al.*, 1996). Phenolics and polyphenols present in the plants are known to be toxic to microorganisms (Mason and Wasserman, 1987). Phytochemicals with direct impacts on the vertebrate reproduction provide an obvious and a compelling system for studying evolutionary toxicology (Lambert and Edwards, 2017). Besides, due to the presence of antimicrobial substances they provide a source of inspiration for novel drug compounds as plant-derived medicines have made significant contribution to human health.

4. Conclusion

The studying of the antioxidant properties and bioactive compounds of the wheat varieties has justified the traditional use of plants in curing diseases. The ethanol extracts of the two tested varieties showed abstemiously-high antioxidant effects. The results revealed that the DPPH free radical scavenging assay plant extracts showed 50% scavenging ability compared with the standard antioxidant (BHT). For further investigation of safe, potent and natural source of diseases and phytochemical constituents of wheat and other plants, this technique and protocol will be helpful for researcher.

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References

Bhattacharjee B and Islam SMS. 2015. Assessment of antibacterial and antifungal activities of the extracts of Rhynchostylis Retusa Blume- A Medicinal Orchid**.** *World J Pharmacy Pharma Sci***., 4(2): 74-87.**

Bhattacharjee B, Islam T, Rahman Z and Islam SMS. 2015. Antimicrobial activity and phytochemical screening of whole plant extracts of *Vanda tessellata* (Roxb.) Hook. Ex. G. Don. *World J Pharmacy Pharma Sci.*, **4(1)**: 72-83.

Burkill HM. 1988. **The Useful Plants of West Tropical Africa**. 2nd Edition, *Royal Botanical Garden, Kew*., **5**: 237-238.

Chatha SAS, Hussain AI, Bajwa JR and Sagir M. 2006. Antioxidant activity of different solvent extracts of rice bran at accelerated storage of sunflower oil. *J Food Lipids.*, **13**: 424-433.

Doughari JH. 2006. Antimicrobial activity of *Tamarindus indica* Linn. *Trop J Pharma Res.*, **5(2)**: 597-603.

Fleischman EF, Kowalski RJ, Morris CF, Nguyen T, Li C, Ganjyal G and Ross CF. 2016. Physical, textural, and antioxidant properties of extruded waxy wheat flour snack supplemented with several varieties of bran, *J Food Sci.*, **81(11)**: E2726-E2733.

Giordano D, Locatelli M, Travaglia F, Bordiga M, Reyneri A, Coisson JD and Blandino M. 2017. Bioactive compound and antioxidant activity distribution in roller-milled and pearled fractions of conventional and pigmented wheat varieties, *Food Chem.*, **233**: 483-491.

Lambert MR and Edwards TM. 2017. Hormonally active phytochemicals and vertebrate evolution. *Evolu Appl.*, **10(5)**: 419-432.

Li Y, Maa D, Suna D, Wanga C, Zhangc J, Xiea Y and Tiancai G. 2015. Total phenolic, flavonoid content, and antioxidant activity of flour, noodles, and steamed bread made from different colored wheat grains by three milling methods. *The Crop J.*, **3**: 328-334.

Mariod AA, Adamu HA, Ismail M and Ismail N. 2010. Antioxidative effects of stabilized and unsterilized defatted rice bran methanolic extracts on the stability of rice bran oil under accelerated conditions. *Grasasy Aceites*., **61**: 409-415.

Mason TL and Wasserman BP. 1987. Inactivation of red beet beta-glucan synthase by native and oxidized phenolic compounds. *Phytochem.*, **26**: 2197-2202.

Mellen PB, Walsh TF and Herrington DM. 2008. Whole grain intake and cardiovascular disease: a meta-analysis, *Nutr Metab Cardiovas Diseases* (Elsevier). **18**: 283-290.

Morshed S and Islam SMS. 2015. Antimicrobial activities and phytochemical screening of corn (*Zea mays* L.) Silk. *SKUAST J Res.*, **17(1)**: 8-14.

Nithya TG, Vidhya VG, Sangeethaand K and Prakash V. 2011. Photochemical screening of a polyherb vallarai chooranam. *Int J Drug Formul Res.*, **2**: 294-301.

Nystrom L, Makinen M, Lampi AM and Piironen V. 2005. Antioxidant activity of steryl ferulate extracts from rye and wheat bran. *J Agr Food Chem.*, **53**: 2503-2510.

Okezie IA. 1999. Free radical, oxidative stress, and antioxidants in human health and disease, *The J Amer Oil Chemists' Soc.*, **75**: 199-212.

Paul SR, Sayeed MR and Hakim ML. 2017. Antibacterial and cytotoxic activity of the bark of *Phoenix paludosa* in different solvents. *Jordan J. Biol Sci.*, **10(3)**: 213-217.

Shahidi F. 1997. **Natural antioxidants: An Overview, Natural antioxidant, Chemistry, Health Effects and Applications**; Champaign, IL, USA: AOCS press. pp 44-46.

Siddhuraju PA and Becker K. 2003. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agro climatic origins of Drum stick tree (*Moringa oleifera* Lam.) leaves. *J Agr Food Chem.*, **51**: 2144-2155.

Sikwese FE and Duodu KG. 2007. Antioxidant effect of a crude phenolic extract from sorghum bran in sunflower oil in the presence of ferric ions. *Food Chem.*, **104**: 324-331.

Trease GE and Evans WE. 2002. **Pharmacognosy**. 15th eds., W.B. Saunders Company Limited, London. p 585.

Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, Tanaka T and Iinuma M. 1996. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*. *J Ethnopharma*, **50(1)**: 27-34.

Wu XL, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE and Prior RL. 2004. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States, *J Agr Food Chem.*, **52**: 4026-4037.

Yu L, Haley S, Perret J, Harris M, Wilson J and Qian M. 2002. Free radical scavenging properties of wheat extracts, *J Agr Food Chem.*, **50**: 1619-1624.

Zuo Y, Wang C and Zhan J. 2002. Separation, characterization, and quantification of benzoic and phenolics antioxidants in American Cranberry fruit by GC-MS, *J Agr Food Chem.*, **50**: 3789-3794.