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Antioxidant activities and total phenolic contents in *Triticum turgidum* L. and *Triticum isphahanicum* Heslot

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Abstract : The total phenolic contents and antioxidant activities of tetraploid wheat samples (*Triticum turgidum* L. subspecies and *Triticum isphanhanicum* Heslot)were evaluated to point out a potential source of natural antioxidants. Antioxidant activity assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH)radical scavenging capacity assay ranged from 45.15% to 72.3%. The highest level was observed in *Triticum turgidum* L.ssp. *turgidum* Var. *mirabile* Korn. Antioxidant activity measured by reducing power assay ranged from 0.02 to 0.42 mg/mL.*Triticum turgidum*L. ssp. *turgidum*Var. *Mirabile* Korn.demonstrated the most value of reducing power. The total phenolic contents in extracts ranged from 61.79 to 96.72 mg GAE/g. The highest level was observed in *Triticum turgidum* L. ssp. *dicoccum*. The total phenolic content was positively correlated with DPPH antioxidant activity, but negatively correlated with reducing power antioxidant activity.
Keywords : antioxidant activity, total phenolic contents, reducing power, *Triticum turgidum*, *Triticum isphahanicum.*

Introduction

Wheat is one of the most commonly consumed crops and an important part of the human diet[1,2].Wheat is primarily used to provide the significant amount of energy and proteins[3]. In addition, wheat grain is a good source of various vitamins, minerals, and antioxidants[4]. Studies revealed that regular consumption of cereals, especially wheat grains can reduce the risk of some chronic diseases such as type-2 diabetes, obesity, cardiovascular disease, and colon cancer[5,6,7]. These health profits are related to the content of phytochemical compounds[8]. It is believed that among these phytochemical compounds antioxidants play an important role in health increasing and illness decreasing effects[9]. Researchers focus on natural antioxidants in plants especially cereals because they are assumed to be less toxic than synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxyltoluene (BHT). These synthetic antioxidants are assumed to be carcinogenic and causing liver illness[10]. The antioxidant capacity of wheat is higher than those of other cereals such as rice[11,7].

Wheat grains also contain several phenolic compounds such as phenolic acids, flavonoids and proanthocyanidins[12]. They occur in wheat grains in 3 forms, including soluble free phenolic acids, soluble phenolic acids conjugated to low molecular components such as sugars, and phenolic acids bound to plant cell wall[13,14,9]. Phenolic compounds of wheat are mainly derived from benzoic and cinnamic acid[13]. Because of their chemical structure, they are able to scavenge or neutralize free radicals by donating electrons[15] and as a result to decrease oxidative damage to DNA and proteins[16].

Antioxidant capacities and the phenolic compounds in wheat have been studied to investigate their health beneficial properties[7,17,18,9,19]. Although hexaploid wheat (e.g. *Triticum aestivum* L.) has been greatly studied to evaluate antioxidants and phenolic compounds, insufficient attention has been paid to tetraploid wheat[18].

The purpose of this study was to determine the antioxidant capacity as measured by the 2,2-diphenyl-1-picrylhydrazyl(DPPH) scavenging assay, the reducing power assay, and total phenolic contents by the Folin–Ciocalteu reagent method in *Triticum turgidam* L. And *Triticumi sphahanicum* Heslotaccessions.

Materials and methods

Wheat samples

Five accessions of tetraploidwheat (*Triticum turgidum* L. ssp.*polonicum, Triticum turgidum* L.ssp.*turgidum* Var. *mirabile* Korn., *Triticum turgidum* L. ssp. *Turgidum* Var. *psudomirabile* Mihi., *Triticumis phahanicum* Heslot ,and *Triticum turgidum* L.ssp.*dicoccum*) were selected for the study among the species largely cultivated in Iran, and provided from Kharazmi University collection. Plants were grown in an experimental field located in Kharazmi University, Karaj, Iran (35º 51' 80" N, 50º 54' 78" E) in November 2011.

Each sample was grown in 10 m^2 plots in a randomized block with three replicates, in the relative selected area. Climatic conditions (rainfall and temperature) were registered in the site along crop year and in particular during the grain filing period (May – June). During this period, the minimum and maximum temperatures were -1 ºC and 33 ºC, respectively. The mean amounts of rainfall were 172.3 mm (November – May) and 69.9 mm (May – June); therefore, plants were irrigated from May to June every 2 weeks.

After harvesting, wheat grains were ground using a laboratory mill (IKA, Staufen, Germany) to particle size 1 mm and the resulting samples were stored at -20 ºC further use.

Preparation of extracts

A 10 g Wheat grain milling fraction was added to 100 mL of 96% ethanol. After that, the mixture was shaken at room temperature for 1 hour. The procedure was repeated twice. Extracts were mixed and then dried using a vacuum-evaporator. The produce was estimated based on the wet mass of the samples. The dried extract was dissolved in 10 mL of 96% ethanol and used for the further investigation on antioxidant activity[20].

DPPH radical scavenging assay

DPPH radical scavenging assay was estimated according to the protocol described by Zhu et al.[21]. Briefly, 2mL of a freshly prepared DPPH solution (0.1 mM, in ethanol) was mixed with 2 mL of the extracts at various concentration (2.5, 5, 7.5, 10, and 12.5 mg/mL). The mixture was shaken and incubated in the dark at room temperature for 1 hour. Then, the absorbance was read spectrophotometrically at 517 nm against a blank solution (Shimadzu, Tokyo, Japan). Controls were prepared in the same way for the test group with the exception of the replacement of the corresponding extraction solvent instead of antioxidant solution. The following formula was used to estimate the inhibition of the DPPH radical by the sample:

$$\frac{\text{Abs.of control} - \text{Abs.of sample}}{\text{Abs.of control}} \times 100\% = \text{DPPH scavenging activity}$$

Reducing power

The reducing power of wheat extracts was measured spectrophotometrically according to the protocol described by Atmani et al.[22]. A 1 mLofthe extracts at various concentrations (5, 10, and 15 mg/mL) was mixed with phosphate buffer (2.5 mL; l0.2 M, pH6.6) and potassium ferrocyanate(1%, 2.5 mL). The mixture was incubated at 50 ºC for 20 minutes. Then, trichloroacetic acid (10%, 2.5 mL) was added to the mixture. After that,the mixture centrifuged at 88g over 10 min. Distilled water (2.5 mL) and ferric chloride (0.5 mL) were added tothe supernatant. The absorbance was measured spectrophotometrically at 700 nm (Shimadzu, Tokyo, Japan).

Determination of total phenols contents

The total phenolic contents (TPC) of the wheat extracts were estimated by Folin-cicalteu method[23] with some modifications. Briefly, 0.1 mL of the extract was mixed with 0.2 mL of Folin-cicalteu reagent (Sigma Aldrich, Munich, Germany) and 2 mL of de-ionized water. Then, 1 mL of sodium carbonate (15%) was added to the mixture, and then vortexed. After a 2 hour reaction, the absorbance of the mixture was measured at 765 nm with a UV–visible spectrophotometer (Shimadzu, Tokyo, Japan). Gallic acid(0, 50, and 100 mg/L) was used as a standard reference, and the results were expressed as milligrams of gallic acid equivalents (GAE) per gram of the sample (mg GAE/g sample).

Statistical analysis

The data obtained in this study were expressed as means ± SD of three replicates. The data were tested by one-way ANOVA, followed by the Fisher's least significant difference (LSD) post hoc test.P-values< 0.05 were considered as statistically significant. Pearson correlation coefficients were calculated for pairs of biochemical parameters. Statistical calculations were conducted using the IBM SPSS version 20 software.

Results and discussion

Antioxidant activity assay

Scavenging the stable DPPH radical is widely used to determine of antioxidant activity in different fruits, vegetables and in particular cereals[24,25]. DPPH scavenging activity assay is a suitable method to estimate antioxidant activity. It measures the activity of the sample against DPPH. In fact, DPPH is a stable organic nitrogen radical with an absorption band at 517 nm[26]. When it accepts an electron or a free radical species, it loses absorption and consequently purple color of solution changes to yellow[26,27]. In this study, the wheat extracts demonstrated DPPH scavenging activities in a concentration-dependent way. As indicated in Figure 1, ssp. *turgidum* Var. *mirabile* showed the highest DPPH radical scavenging activity at all concentrations. While ssp. *polonicum* showed higher DPPH scavenging activity at lower concentrations (2.5 to 5 mg/mL), ssp. *Dicoccum* showed stronger DPPH scavenging capacity at higher concentrations (7 to 12.5 mg/mL) than ssp. *polonicum* did. In addition, ssp. *Turgidum* Var. *pseudomirabile* showed the lowest DPPH scavenging activity among all the wheat extracts. Overall, the mean antioxidant activity assessed by the DPPH radical scavenging capacity varied from 45.15% to 72.03 % (Table 1). The scavenging activity values decreased in the following order: ssp. *turgidum* Var. *mirabile*> ssp. *dicoccum*> ssp. *polonicum*>*T. isphahanicum*> ssp. *turgidum*V ar.*pseudomirabile*.There were no significant differences (*P*< 0.05) in the DPPH radical scavenging activity values among the extracts with the exception of thessp. *turgidum* Var. *mirabile* and ssp. *turgidum* Var. *pseudomirabile*. Pasqualone et al.[18]reportedthatantioxidant activity values ranged from 47.5% to 81.7%,in different tetraploid samples. The results were compatible with those of this study; although they used different extraction protocols. According to Pasqualone et al.[18], the highest antioxidant activities were measured in ssp. *dicoccum* (81.7%) and ssp. *dicoccoides* (80.0%). Mpofu et al.[28] observed up to 15.06 % antioxidant activity in hard spring wheat samples (*Triticum aestivum* L.). According to Fardet el al.[29],cereals have a considerable antioxidant activity in vitro. DPPH assay showed that the average antioxidant activity of cereals was higher than those of common fruits and vegetables, but lower than those of common berries[30].

Reducing power

The reducing power of the extract is another useful method to evaluate antioxidant activity. In this method, Fe^{3+} reducestoFe^{2+}, and whereby the yellow color of the solution changes to various colors of green or blue. The antioxidant solution can be assayed by absorption of blue color at 700 nm[31]. The reducing power of the various wheat extracts is presented in Figure 2. All extracts showed concentration-dependent and acutely increasing reducing power with the exception of ssp. *turgidum* Var. *pseudomirabile*. At 5 mg/ml concentration, ssp. *polonicum*, ssp. *Dicoccum*a nd ssp. *turgidum* Var. *mirabile* showed almost equal values (0.22, 0.20, and 0.19 mg/mL absorption, respectively). While ssp. *dicoccum* showed the highest reducing power at 5 mg/mL concentration,ssp. *turgidum* Var. *mirabile* demonstrated the highest reducing power at10 mg/mL concentration. The mean reducing power of the extracts ranged from 0.02 to 0.42 mg/ml (Table 1). The values decreased in the following order: ssp. *polonicum*>ssp. *turgidum* Var. *mirabile*> ssp. *dicoccum*>*T. isphahanicum*> ssp. *turgidum* Var. *pseudomirabile*. There were no significant differences (*P*< 0.05) in the reducing power values among the extracts except ssp. *turgidum* Var. *pseudomirabile*.

$IC_{50}$of DPPH and reducing power`

The IC_{50} value is the concentration of the extract sample necessary to lead 50% inhibition measured by linear regression analysis[31]. A lower IC_{50} value shows a higher radical scavenging activity. TheIC_{50}values of DPPH scavenging activity in the extracts ranged from 0.11 to 21.3 mg/mL (Table 2). It was found that ssp. *turgidum* Var. *mirabile* had the strongest DPPH radical scavenging activity. It is interesting that the other cultivar, ssp. *turgidum* Var. *pseudomirabile*, had the weakest DPPH radical scavenging activity. TheIC_{50} values of DPPH revealed that scavenging activity decreased in the following order:ssp. *turgidum* Var. *mirabile*> ssp. *polonicum*> ssp. *dicoccum*>*T. isphahanicum*> ssp. *turgidum* Var. *pseudomirabile*.

The IC_{50} values of reducing power in the extracts ranged from 11.87 to 403.66 mg/mL (Table 2). The order of IC_{50} of reducing power was completely compatible with that of the DPPH radical scavenging activity. There were no significant differences ($P< 0.05$) in the reducing power values among the extracts with the exception of the ssp. *turgidum* Var. *pseudomirabile*. According to Sedej et al.[20] the IC_{50} of the reducing power in buckwheat flour and wheat flour were 2.5 and 10.11 mg/mL, respectively. These result showed that buckwheat flour had higher reducing power compared to the tetraploid wheat in the study.

Total phenolic contents

Total phenolic contents (TPC) is another convenient method to evaluate the possible source of antioxidants in samples. In this study, the total phenolic contents of the wheat extracts were measured according to the Folin-Ciocalteu method. The values were obtained from the calibration curve (y = 0.0075x + 0.0012, R^2 = 0.9989, x is the absorbance; y is the concentration of gallic acid solution, mg/ml) and demonstrated as gallic acid equivalents (mg gallic acid/g dried extract).

In this study, the values ranged from 41.79 to 96.71 mg GAE/g (Table. 1). The highest TPC was detected in ssp. *dicoccum* (96.71mg GAE/g) and the lowest in ssp. *polonicum* (41.79 mg GAE/g). In overall, the total phenolic contents values decreased in the following order: ssp. *dicoccum*>*T. isphahanicum*> ssp. *turgidum* Var. *pseudomirabile*> ssp. *turgidum* Var. *mirabile*> ssp. *polonicum*. There were significant differences (P< 0.05) in the total phenolic contents among the extracts. These results were lower than those reported by Giambanelliet al.[32] and higher than those reported by Ciccorittiet al.[33].Giambanelliet al.[32] showed that the total phenolic contents in ssp. *dicoccum* ranged from 831.6 to 1248.4 mg/kg Dm. According to Cicoritti el al.[33], the total phenolic contents were measured 74 mg cathechin / kg in ssp. *dicoccum*, while the values were evaluated 141 and 144 mg cathechin/kg in ssp. *durum* and *T. aestivum*, respectively. In fact, lack of standardized methods to extract, estimate and explain the results makes comparisons with other studies relatively difficult[34,35,36].Environmental factors can also can affect the grain size and consequently the phenolic contents[28]. In addition, climatic conditions such as dry or rainy weather during the seed growth may lead to modify phenolic contents[37]. Bellato et al.[38] showed the influence of environment and genotype on the total phenolic contents of ssp. *durum*.

Correlation analyses between antioxidant activity and total phenolic contents in wheat samples

To confirm the major antioxidant capacity contributors in wheat extracts, the antioxidant activity evaluated by DPPH assay and reducing power was correlated with the total phenolic contents measured by Folin-ciocalteu method.

The total phenolic content was positively correlated with the DPPH antioxidant activity (r = 0.913; $P< 0.01$; data are not shown) and negatively correlated with reducing power (r = 0.7; $P< 0.01$; data are not shown). However, there was no correlation between DPPH and reducing power of wheat extracts. Positive correlation between the total phenolic contents and DPPH antioxidant activity was also reported by other researchers[39,17,22,20,40,41].Thispositive correlation remarks the antioxidant effect of phenolic compounds[18]. Negative correlation between reducing power and total phenolic content suggested a different trend in antioxidant capacity between DPPH and reducing power, for instance, between nitrogen radical scavenging and ferric-reducing capacity[40].On the other hand, Atmani el al.[22] and Sedejet al.[20] reported a positive correlation between DPPH antioxidant activity and reducing power. These resultswere compatible with this study findings.

Table 1 DPPH scavenging activity, reducing power and total phenolic contents of the wheat extracts.

Samples	DPPH Antioxidant activity (%)	Reducing power (mg/mL)	Total Phenol (mg GAE/g)
Triticm turgidum ssp. *polonicum*	63.87 ± 7.26 a	0.42 ± 0.01a	41.79 ± 2.3 a
Triticum turgidum ssp. *turgidum* Var. *mirabile*	72.03 ± 8.41 b	0.41 ± 0.01 a	52.19 ± 3.31 b
Triticum turgidum ssp. *turgidum* Var. *pseudomirabile*	45.15 ± 1.30 c	0.02 ± 0.00 b	60.37 ± 3.27 c
Triticum turgidum ssp. *dicoccum*	65.04 ± 10.13 a	0.39 ± 0.01 a	96.72 ± 4.38 d
Triticum isphahanicum	61.48 ± 8.68 a	0.32 ± 0.04 a	84.59 ± 4.24 e

Values are maeans ± SD, n=3. Antioxidant activity is expressed as % discoloration of 2,2-diphenyl-1-picrylhydrazyl (DPPH). Means followed by the same letter in the same column are not significantly different ($P < 0.05$).

Table 2 IC_{50} in DPPH radical scavenging activity and reducing power of the wheat extracts.

Samples	IC_{50} of DPPH radical (mg/mL)	IC_{50} of reducing power (mg/mL)
Triticm turgidum* ssp. *polonicum	**0.33 ± 1.54 a**	11.92 ± 0.23 c
Triticum turgidum* ssp. *turgidum* Var. *mirabile	**0.11 ± 1.12 a**	11.87 ± 0.36 c
Triticum turgidum* ssp. *turgidum* Var. *pseudomirabile	**21.3 ± 0.02 c**	403.66 ± 128.2 d
Triticum turgidum* ssp. *dicoccum	**2.09 ± 0.41 b**	12.85 ± 0.76 c
Triticum isphahanicum	2.43 ± 0.45 b	15.19 ± 2.38 c

Values are means ± SD, n=3. Means followed by the same letter in the same column are not significantly different ($P < 0.05$); DPPH: 2,2-diphenyl-1-picrylhydrazyl

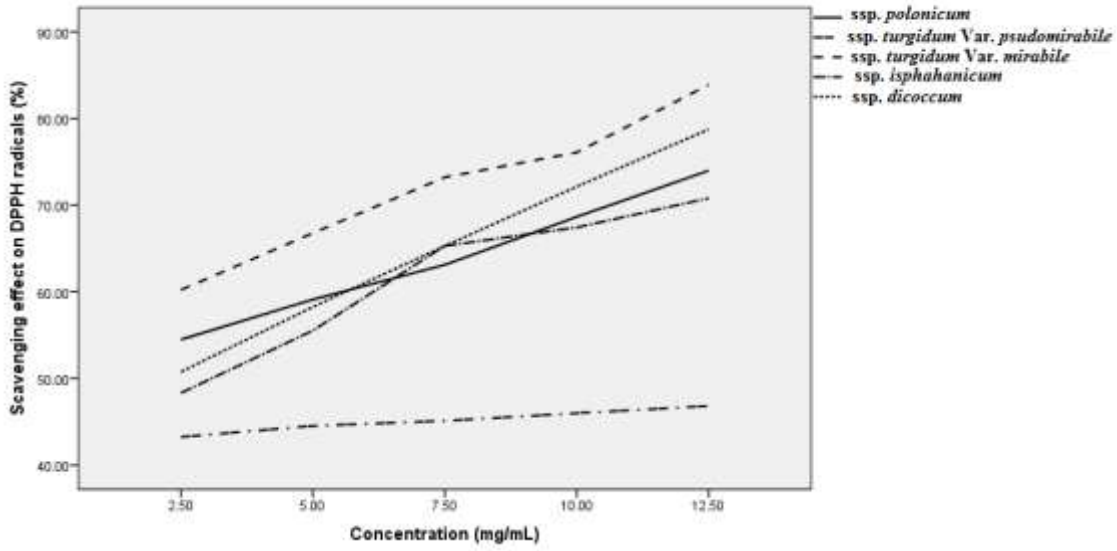

Figure 1 DPPH radical scavenging activities of the wheat extracts.

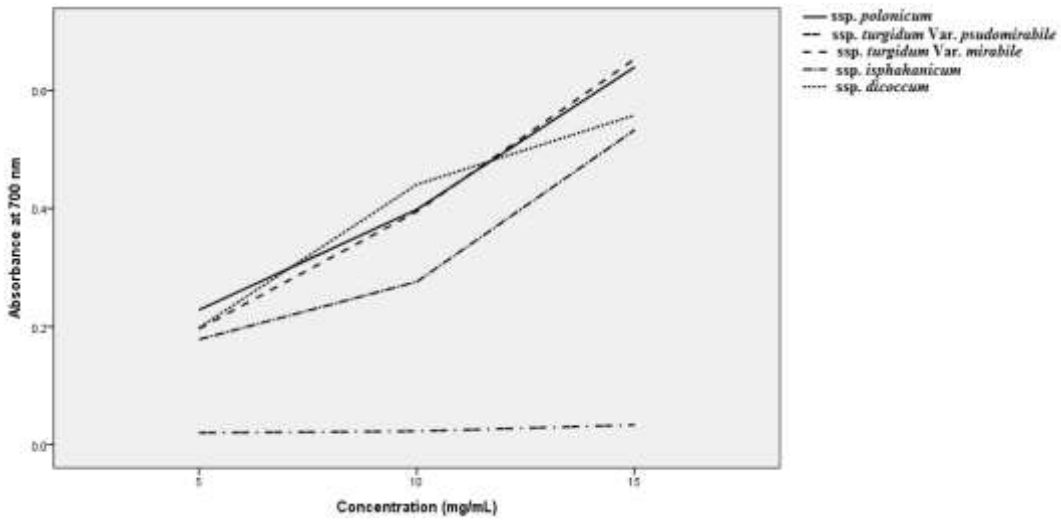

Figure 2 Reducing power of the wheat extracts.

Conclusions

In this study, all the wheat extracts demonstrated considerable antioxidant activity in the DPPH radical scavenging assay and the reducing power assay with the exception of *Triticum turgidum* ssp. Var. *pseudomirabile*. Two samples, *Triticum turgidum* ssp. *turgidum*Var. *mirabile*. And *Triticum turgidum* ssp.

polonicum, showed the highest antioxidant activity among the extracts.All the extracts could be considered as a valuable source of total phenolic compounds. *Triticum turgidum* ssp.*dicoccum* and *Triticum ishphahanicum* showed the highest total phenolic contents among wheat extracts. The results of this study suggested that wheat extracts could be served as a beneficial source of natural antioxidants.

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