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Phytochemical Screening and Radical Scavenging Activity of Whole Seed of Durum Wheat (*Triticum durum* Desf.) and Barley (*Hordeum vulgare* L.) Varieties

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Abstract

Three durum wheat (*Triticum turgidum* L. var. *durum*) cultivars, namely Bousselam, Vitron, and Gaviota *durum*, and one barley genotype (*Hordum vulgare* L.), Fouara, grown under semi-arid conditions were compared for their total phenolic and flavonoids content and antioxidant activities. Antioxidant activity was tested using DPPH radical scavenging assay method. The phytochemical screening revealed the presence of tannins, flavonoids, coumarins, saponins and phenolic compounds in each variety seeds. The results of the present study indicate significant differences among the evaluated varieties in terms of total phenolic and flavonoid contents and for radical scavenging capacity. Among the tested varieties Gaviota durum showed high total phenolic (95.32± 0.27 µg /mg) and flavonoid content (78.80 ± 0.27 µg/mg) and an intermediate radical scavenging capacity. While barley variety Fouara expressed high radical scavenging capacity (54.8% ±0.34) and intermediate total phenol and flavonoids contents. The tested durum wheat and barley varieties possessed varying but meaningful antioxidant activities which were not significantly correlated to their phenol and flavonoid contents. It is necessary to ensure that increased bioactive components in grains are combined with good agronomic performance, high grain yield and high quality for processing. The results of the present study should have significant implications for plant breeders as well as for grain and food processors.

Keywords: Wheat, Antioxidant, Barley, Flavonoids, Total phenolic content, DPPH.

1. Introduction

Cereals are an important component of the human diet, and are used in the production of many food products, providing energy based on their high protein and carbohydrate contents (Sarwar *et al*., 2013). They are also characterized by a high amount of insoluble and soluble bioactive components like fibers, vitamins, minerals, unsaturated fatty acids, tocopherols, lignans, flavonoids and phenolic acids (Okarter *et al.*, 2010). When facing oxidative conditions, caused by stresses such as heat, drought, UV radiation, chemicals or pathogens attacks, plants produce secondary metabolites as a self-protection mechanism to intercept oxidative reactions generating free radicals and converting them into harmless molecules (Manach *et al.*, 2004). In fact, reactive oxygen species and free radicals are constantly formed in the organism body by normal metabolic actions. Their effects are opposed by a balanced system of antioxidants and enzymes defenses. Unbalance between these two systems causes oxidative stress, which can lead to cell injury and death (Romano *et al.*, 2010).

Since synthetic antioxidants are suspected of being carcinogenic (Ratnam *et al*., 2006), much attention has been given to naturally occurring antioxidants, which are able to inhibit oxidative chain reactions within tissues (Nsimba *et al.*, 2008). According to Perez-Jimenez *et al.* (2008), increased intake of antioxidants rich food is associated with a lower risk of cardiovascular and cancer diseases. Naczk and Shahidi (2006) mentioned that phenolic compounds are employed in the treatment of cardiovascular diseases. Consumption of cereals containing high levels of antioxidants, mostly coming from phenols, has been recommended (Ward *et al*., 2008).

Anson *et al.* (2008) mentioned that the major components with antioxidant activity in wheat belong to the group of phenolic acids, which are mostly found in the bran, suggesting the use of wheat grain as whole instead of refined. Traditionally, wheat grain is milling to obtain the refined white flour by removing the bran. According to Dvorakova *et al.* (2010), barley is an excellent source of phenolic acids, flavonoids, tannins, proanthocyanidins and

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amino phenolic compounds. Adom *et al*. (2005) reported that antioxidant content depends on the cereal species, varieties, environmental conditions and treatment type.

The aim of the present study was to compare phenolic and flavonoid contents and their antioxidant activity in the seeds of three durum wheat (*Triticum turgidum* L. var. *durum*.) and one barley (*Hordeum vulgare* L.) varieties produced under semi-arid conditions.

2. Materials and Methods

2.1. Plant Materials

Three durum wheat (*Triticum turgidum* L. var. *durum*) cultuvars, namely Bousselam, Vitron, and Gaviota *durum*, and one barley variety (*Hordum vulgare* L.), Fouara, were used as plant material. Seeds were generously provided by Ain Babouche Cereals and Food Legumes Cooperative (Oum El Bouaghi, Algeria). These varieties were authenticated based on seed increase certificates delivered in June 2016, by the National Center of Seed Control and Certification, Khroub laboratory, Algeria (CNCC, Khroub, Algeria). Pedigree and cross origin of the durum wheat varieties were reported by Hamli *et al*. (2015**)** and the one of barley variety was reported by Bensemane *et al*., (2011). Seeds of the four varieties were cleaned and milled to a fine powder with cyclotec sample mill to pass through a 0.5 mm sieve.

2.2. Extracts Preparation

Extraction of the ground material was conducted according to Sultana *et al.* (2008) with some modifications. 10 grams of milled wheat and barley seed samples were extracted for 24 h with 100 ml of 70% aqueous methanol (v/v) at ambient temperature, in amber flasks in a shaking water bath. The supernatant was transferred to volumetric flask and the pellet was re-extracted for 24 h with another 100 mL of 70% aqueous methanol. Supernatants from both extractions were combined and the residues were separated by filtering through Whatman filter paper (No. 1, Whatman International Ltd., Kent, England). Methanol was evaporated using a rotary evaporator (HAHNVAPOR) under reduced pressure and mild temperature (<40°C).The combined crude extracts were weighed, and then kept in the dark until used for of various antioxidant bioassays and for determination of total phenolic, flavonoid content, and DPPH radical scavenging activity.

2.3. Qualitative Screening

2.3.1. Alkaloids and Flavonoids Screening

The presence of alkaloids was tested using Mayer reagent and according to the procedure described in the literature (Edeoga, 2005). Briefly, a 5 mL sample of the crude plant extract was added to a mixture of mercuric chloride and potassium iodide. The appearance of a white pale precipitate indicated the presence of alkaloids.

The presence of flavonoids was tested using the methods described by Edeoga (2005). Few drops of aluminium chloride solution (1 % $AlCl_3$ v/wt) were added to 5 mL of crude extract. The appearance of a yellow color indicated a positive result.

2.3.2. Tannins and Saponins Screening

The presence of tannins was tested by adding few drops of dilute iron chloride ($FeCl_3$, 2 %) to 5 mL of crude extract. According to Karumi *et al.* (2004), formation a dark blue colored precipitate indicated the presence of tannins. The presence of saponins was tested according to the procedure described by Karumi *et al.* (2004). Briefly, a mixture containing 5 ml of the crude extract in 10 ml distilled water was shaken vigorously for 2 minutes. The formation of forth indicated the presence of saponins.

2.3.3. Coumarins Screening

The presence of coumarins is based on the Keller-Kiliani reaction **(**Edeoga, 2005). 1 ml of crude extract was dissolved in 5 ml of acetic acid containing one drop of ($FeCl_3$) solution and 5 mL of sulfuric acid were added to the mixture. The presence of coumarins is indicated by the formation of two phases, one red-brown colored suggesting the presence of acetic acid and the other blue-green colored, suggesting the presence of sulfuric acid (Edeoga, 2005).

2.4. Quantitative Analyses

2.4.1. Total Phenolic Content

The Total Phenolic Content (TPC) in the methanolic crude extract was determined using the Folin-Ciocalteu's reagent according to the method described by Singleton *et al.* (1999). The extract (0.2 mL) diluted ten times was left; react with 1 ml of the reagent of Folin-Ciocalteu, and then the mixture was neutralized with 0.8 ml of 20% sodium carbonate (w/v) solution. The mixture was then incubated at ambient temperature for 30 min after which, the absorbance was measured at 760 nm using a helios spectrophotometer (thermo spectronic). A standard curve was prepared using gallic acid standard solutions of known concentrations, yielding a calibration curve having the following form: Y=0.0127X-0.0106 with a coefficient of determination $R^2 = 0.9988$, where Y= absorbance, and X= GAE concentration in µg/ml. The results are expressed as µg of gallic acid equivalent per mg of crude extract sample (µg GAE/mg sample). For each sample, three replicate assays were performed.

2.4.2. Total Flavonoid Content

Flavonoid contents of wheat and barley seed crude extract were assayed using the aluminum chloride colorimetric method described by Ordonez *el al.* (2006). Crude extract (0.5 mL) was mixed with 0.5 mL of 2 % methanolic solution of aluminum chloride (v $AlCl_3$/v Methanol). After incubation at room temperature for 10 min, the absorbance of the reaction mixture was measured at 420 nm with a helios spectrophotometer (thermo spectronic). The total flavonoid content was determined using a standard curve obtained from various concentrations of quercetin and expressed as µg of Quercetin Acid Equivalent (QAE) per mg of dry matter (µg QAE/mg). Calibration curve had the following form: Y=0.0334X-0.1031 with a coefficient of determination $R^2 = 0.9382$, where Y= absorbance, and X= quercetin concentration µg/mL.

2.4.3. Total Tanins Content

Total tanins content in extracts is carried out according to the method of Heimler *et al.* (2006). For 50 µL of the crude extracts we add 500 µL of vanillin (4% in methanol) and 1.5 mL of concentrated HCl. The mixture is incubated during 20 min and the absorbance was measured at 500 nm with a helios spectrophotometer (thermo spectronic). The total tanins content was determined using a standard curve obtained from various concentrations of catechine and expressed as µg of Catechine Acid Equivalent (CAE) per mg of dry matter (µg CAE/mg). Calibration curve had the following form: Y=0.133X-0.018 with a coefficient of determination $R^2 = 0.966$, where Y= absorbance, and X= catechine concentration in µg/ml.

2.4.4. Antioxidant Activity

The antioxidant capacity of the crude seed wheat and barley extracts was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the method described by Huang *et al.* (2005). Samples of 0.2 mL of crude extracts were mixed with 1.8 mL of DPPH solution (0.04 g DPPH in 100 mL methanol) and incubated for 30 min at room temperature. Decline in absorbance of the reaction mixture at the end of the incubation period was measured using a helios spectrophotometer (thermo spectronic) at 517 nm. Free radicals scavenging capacity was calculated according to the following equation:

$$\mathbf{DPPH*scavenging}(\%) = \frac{\mathbf{absorbance_{control}} - \mathbf{Absorbance_{sample}}}{\mathbf{Absorbance_{control}}}\mathbf{x}100$$

2.5. Statistical Analyses

Data were subjected to an analysis of variance of randomized design with a single factor and three replications. Student-Newman-Keulstest was carried out to assess significant differences between treatment means, using SAS version 9.1.3., (2011). Spearman rang correlation coefficients (rs) were calculated to determine the relationships between measured variables at the 5% probability level of significance.

3. Results and Discussion

3.1. Qualitative Screening

Qualitative screening was performed in order to check for the presence/absence of secondary metabolites. The screening revealed the presence of tannins, flavonoids, coumarins saponins and phenolic compounds in each variety seeds .These results suggested that quantification of the targeted compounds could be carried out (Table 1).

Table1. Phytochemical screening of barley and wheat varieties

Parameters	Flavonoids	Tannins	Alkaloids	Coumarins	Phenols	Saponins
Wheat varieties	+	+	-	+	+	+
Barley	+	+	-	+	+	+

(+) = indicates presence of compounds, (-) = indicates absence of compounds

3.2. Quantitative Screening

3.2.1. Total Phenolic Content

Even though free polyphenols compounds are easily extracted using various solvents, such as methanol, ethanol, acetone, diethyl ether; studies of Ivanisova *et al.* (2014) indicated that antioxidants and polyphenols from cereals may be effectively extracted by methanol at laboratory temperature. Therefore, this solvent was used, in the present study, for the extraction and evaluation of antioxidants and polyphenols present in durum wheat and barley. Data analysis of variance indicated a significant variety effect for total phenolic, flavonoid and the percentage of inhibition (I %) of crude extract of durum wheat and barley seeds and no significant variety effect for tannins contents (Table 2). Mean values of total phenolic content of crude extracts, ranged from 10.63± 0.35 µg /mg, measured in Bousselam variety to 95.32± 0.27 µg /mg, measured in Gaviota durum (Table 3).

Table2. Means squares of the analysis of variance of total phenolic, flavonoid and the percentage of inhibition (I %) of crude extract of durum wheat and barley seeds

Source	DF	Phenols	Flavonoids	Tannins	% I
Variety	3	4688.44**	3853.93**	6.23 **ns**	3045.97**
Error	8	0.07	0.09	0.05	2.77

****** = Significant variety effect at 1% probability. **ns** = no significant effect. I% = the percentage of inhibition= DPPH scavenging activity. DF: degrees of freedom

Table 3. Mean values of total phenolic, flavonoid and tannins contents of crude extracts of durum wheat and barley seeds

Varieties	Phenols (1)	Flavonoids (2)	Tanins	%I (3)
Gaviota durum	95.32^{a}±0.27	78.8^{a}±0.23	4.39^{a} ±0.16	38.17^{c}
Fouara	43.66^{b}±0.27	22.1^{b}±0.34	4.5^{a}± 0.27	54.80^{b}
Vitron	12.56^{c}±0.11	3.3^{c}±0.44	2.40^{b} ±0.23	0.18^{e}
Bousselam	10.63^{d}±0.35	2.7^{c}±0.06	1.62^{c} ±0.22	36.55^{d}
Ascorbic acid	-	-	-	88.00^{a}

(1) µg of gallic acid equivalent per mg of crude extract sample (µg GAE/mg sample). (2) µg of quercitin acid equivalent per mg of crude extract sample (µg EQ/mg sample). (3) µg of Catechin acid equivalent per mg of crude extract sample (µg EC/mg sample). Means followed by the same letter, within the same column, are not significantly different at 5% level according to the NK'S test. a,b,c and d: homogenized group. I% = the percentage of inhibition= DPPH radical scavenging activity.

Total phenolic content of Gaviota durum was 8.9 fold higher than the one of Bousselam, Vitron being more similar to Bousselam even though significantly different from it.

Fouara, barley variety presented an average value of 43.66 ± 0.27 µg /mg of total phenolic content. The current results are relatively lower than those reported by Amarowicz *et al.* (2002) (92.0 mg/g). Zeilinski and Hozlowoka (2000) as well as Liu and Yao (2007) noted that barley seeds expressed higher amount of phenolic content than common wheat. Comparatively, Abozed *et al.* (2014) reported lower values of total phenolic content

ranging from 3.88 to 4.66 µg /mg in the bran and from 1.78 to 2.57 µg /mg in whole grain of two common wheat varieties. Differences between and within species could originate from genetic factors, geographical and climatic factors, storage conditions and nature of solvent used for extraction.

Also note that despite these cultivars were obtained from the same region, it seems that they did not belong to the same collection period, thus in addition to the genetic factors, other factors including the collection period, drying method, storage conditions, extraction procedure and many other factors may affect the content of secondary metabolites.

3.2.2. Total Flavonoids Content

Mean values of flavonoids content of crude extracts, ranged from 2.70 ± 0.06 µg/mg, average value of Bousselam variety to 78.80 ± 0.27 µg/mg, measured in Gaviota durum (Table 3). Barley variety presented a mean value of 22.10 ± 0.34 µg/mg of flavonoids content. Gaviota durum presented high flavonoid content, 29.1 times fold higher than the flavonoid content of Bousselam, and 3.5 folds higher than barley variety Fouara. Bousselam and Vitron showed similar pattern of flavonoid content.

According to Satheeshkumar *et al.* (2011) flavonoids, based on their structure, are potential antioxidants, since they are able to scavenge, *in vivo* and *in vitro*, practically all known reactive oxygen species. Pinent *et al.* (2008) reported that flavonoids have positive effects on the regulation of glucose homeostasis and anti-inflammatory functions alleviating insulin-mediated chronic diseases, such as insulin resistance. Durum wheat variety Gaviota durum and barley variety Fouara could be recommended for the food industry as natural antioxidant ingredients because of their high level of flavonoid contents.

3.2.3. DPPH Radical Scavenging Activity

Roginsky and Lissi (2005) mentioned that DPPH radical test is based on the ability of this compound to react with hydrogen donor species, mainly polyphenols, and upon receiving proton from extract constituents, it loses its color, which changes from deep violet to yellow. As the concentration of phenolic compounds or degree of hydroxylation of the phenolic compounds increases, their DPPH scavenging activity increases too, correlating directly to the extent of Radical scavenging efficacy of the assessed plant material. Results of the present study show that the order of free radical scavenging activity of crude extracts from seeds of durum wheat and barley varieties was as follows: Fouara barley (54.8% ±0.34) > Gaviota durum wheat variety (38.0% ±0.28) > Bousselam variety (36.9% ±0.41) > Vitron seeds crude extract (0.18 % ± 0.10). Results are summarized in Table 3.

The results of the present study corroborate those of Abozed *et al.* (2014) who reported for two common wheat varieties strong DPPH free radical scavenging activities ranging from 28.07 to 36.82%, for bran samples. Amarowicz *et al.* (2002) reported that barley crude extract was found to afford the strongest free-radical scavenging activities at 43.6% efficacy, which was much stronger than those of the extracts prepared from rye, wheat and triticale caryopses. The results of correlation analyses between the total phenolic content, flavonoid and antiradical activity indicted that only TPC was positively and significantly correlated with flavonoid content (rs = 0.999, $P<0.000$), while these two parameters were not significantly correlated with % I (rs = 0.600, $P>0.050$). These results do not corroborate results reported by Siddhuraju and Becker (2003) who found a significant correlation and commented that antioxidant activity of phenolic compounds is mostly associated with their redox properties which allow them to act as anti-oxidative agents.

4. Conclusion

The results of the present study indicate significant differences among the evaluated varieties for total phenolic and flavonoid contents and for radical scavenging capacity. Among the tested varieties Gaviota durum showed high total phenolic and flavonoid content and an intermediate radical scavenging capacity. While barley variety Fouara expressed high radical scavenging capacity and intermediate total phenol and flavonoids contents. The tested durum wheat and barley varieties possessed varying but meaningful antioxidant activities which, however, were not significantly correlated their en phenol and flavonoids contents. Further studies are recommended to sample a larger set of varieties, grain fractions and solvent types. The results suggested that selected durum and barley varieties may be considered as rich sources of natural antioxidants, which can ideally serve as basis for the development of functional foods designed to improve consumer's health. It is also necessary to ensure that increased bioactive components in grains are combined with good agronomic performance, high grain yield and high quality for processing. The results of the present study should have significant implications for plant breeders as well as for grain and food processors.

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