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Research Paper

Phenolic compound content and antioxidant activity of hydro-ethanolic extracts of durum wheat bran (*Triticum turgidum* subsp. Durum) of some Algerian varieties

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Abstract

The aim of this study is to evaluate the content of durum wheat bran (DWB) of three Algerian varieties in secondary metabolisms such as total phenols, flavonoids and condensed tannins, and to determine their antioxidant power. The experiment was carried out on the following three varieties of durum wheat (*Triticum turgidum* subsp. Durum): Siméto, Chen's and Vitron. The maceration of the wheat bran powder was realized with 80% ethanol. The determination of total phenols was carried out by the folin-ciocalteu method, that of flavonoids by the aluminum trichloride and sodium hydroxide process and condensed tannins by the vanillin method. Antioxidant activity was tested by four methods: free radical scavenging with DPPH, FRAP (Ferric Reducing Antioxidant Power) test, β-carotene bleaching test and the ferrous ion chelating effect (FIC). The total phenol contents (TPC) for the Siméto, Chen's and Vitron varieties are respectively: 1.678±0.051 mg AGE/g DWB, 1.817±0.231 mg AGE/g DWB and 1.813±0.075 mg AGE/g DWB. The concentration of flavonoids (TFC) is: 0.543±0.102 mg CE/g DWB (Siméto), 0.594±0.116 mg CE/g (Chen's) DWB and 0.503±0.077 mg CE/g DWB for Vitron. The content of condensed tannins (CTC) is 1.526±0.193 mg CE/g DWB for siméto, 1.345±0.850 mg CE/g DWB for chen's and 1.587±0.182 mg CE/g DWB for vitron. The antioxidant activity revealed that the antioxidant power with DPPH and FRAP test is less important compared to the bleaching of β-carotene and the chelating effect of ferrous ions (FIC) which gives the same percentage of activity as the standard. This study demonstrates the richness of durum wheat bran in phenolic compounds and antioxidant substances.

Keywords: Wheat bran, *Triticum durum*, phenolic compounds, antioxidant activity, Chelating activity.

Introduction

Cereal-based foods are by far the major source of food, energy, protein, B vitamins and minerals for the current world population[1]. The use of cereals has evolved considerably and is divided between human food, animal feed and non-food applications[2]. Wheat is the world's largest and most important food crop for direct human consumption[3], 45% of durum wheat is grown in Western Asia and North Africa. Wheat is also widely grown in Mediterranean countries, parts of Eastern Europe, the United States, Mexico and parts of Saskatchewan and surrounding areas in Canada. In South America, durum wheat is grown in Chile, Argentina and the Andean regions[4]. In Algeria, cereals in general and wheat (durum and common) in particular are the main staple diet for consumers. They provide more

than 60% of the calorific intake and 75–80% of the protein intake of the food ration[5]. The functional health properties of whole wheat go far beyond its nutritional properties and are mostly derived from the bioactive compounds in the fraction of the outer layer of the seed that represents the bran (a by-product obtained after refining) intended mainly for animal feed. So there are many Studies have shown that the consumption of wheat bran and its products have a beneficial effect on the prevention and risk reduction of chronic diseases such as atherosclerosis, obesity, type2 diabetes, cancer and some gastrointestinal diseases, including diverticular disease, constipation and irritable bowel syndrome[6,7,8] because it has a physiological effect that can be split into nutritional effects from its constituent nutrients; mechanical effects in the gastrointestinal tract due to its fiber content and antioxidant effects arising from its phytochemical constituents[8]. In wheat bran Ferulic acid is a major phenolic acid presented with other phenolic acids such as vanillic acid, p-coumaric acid, syringic acid, caffeic acid, p-hydroxybenzoic acid[9]. The total phenolic acid content in the wheat bran is around 4.5 µg/g and bran ferulic acid is an example of the hydroxycinnamic acids[10].

Bran also comprises water-insoluble fiber involved in the protection of grain and endosperm. This fiber is made of Arabinoxylan (19–25%), Starch (17–29%), protein (14–18%), Lignin (~3%), β-glucans (1–3%), Phytic acid (3–5%), ferulic acid (0.3-5%)[8]. Plant and plant products are being used as a source of medicine for a long time. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, no side effects and economic viability[9]. Phenolic acids and other antioxidants, which are abundant in cereals, can be used more effectively as free radical scavengers as well as reducing and chelating agents for metal ions[11]. However, until now, the majority of studies have been focused on phytochemicals from such sources as vegetables, fruits, and spices, while cereals also accumulate valuable constituents, which might be used in the development and production of healthy dietary ingredients[11]. Also, there are several studies on the phenolic acids contained in wheat, while information on the content of the other polyphenols (lignans, flavonoids) of wheat is poor[12], these show that the wheat bran is a good source of diverse nutrients and antioxidant activity materials such as tocopherols, carotenoids, phenolic acids and phytochemicals[13]. So, the objective of this study is to determine the total phenolic content of durum wheat bran (*Triticum turgidum* subsp. Durum) of three Algerian varieties and to compare their antioxidant activities with many deferent methods.

Materials and Methods

Plant material

Three samples varieties of durum wheat “Triticum turgidum subsp. Durum (Desf.)”: Siméto, Chen’s and Vitron (Table 1)[14-16], were recovered from the National Centre for Seed and Plant Control and Certification of Sidi Bel Abbès, western Algeria. The varieties were harvested during June 2018 in different regions. The first sample from *Mascara* region*:* Latitude: 35.3833, longitude: 0.15 35° 22′ 59″ N, 0° 9′ 0″ E, Altitude: 489 m with a semi-arid dry and cold climate (Köppen classification: BSk). The second sample from *Ain Temouchent* region*:* Latitude: 35.2895, longitude: -1.14099 35° 17′ 22″ N, 1° 8′ 28″ W, Altitude: 245 m with semi-arid dry and cold climate (Köppen classification: BSk), and the third sample from *Adrar* region*:* Latitude: 27.8667, longitude: -0.283333 27° 52′ 0″ N, 0° 16′ 59″ W, Altitude: 279 m with dry and hot desertic climate (Köppen classification: BWh). The three varieties processed in a laboratory mill to obtain refined bran with 1–3 mm dimensions.

Table 1: The durum wheat varieties investigated

species	Varieties	Origin	Pedigree	Crossbreeding
Triticum turgidum **subsp. Durum (Desf.) Husn**	Chen’s	Syria	Ihwa’S’/Bit ’S’CD 26406-3B-2Y-9Y-OM-3Y-OB	CIMMYT-ICARDA
	Siméto	Italy	Capeiti8/Valvona	Italy
	Vitron	Spain	Turkey77/3/Jori-69/Anhinga//Flamingo	CIMMYT-ICARDA

Preparation of extracts

A quantity of 1.5 g of durum wheat bran of each variety has been finely ground and macerated separately in 30 ml of 80% (v/v) ethanol for 24 hours with vigorous agitation. After filtration with wattman paper, the extracts were stored in test tubes at 4°C.

Determination of phenolic compounds

The concentrations of total phenols content (TPC), total flavonoids content (TFC) and condensed tannins content (CTC) in the durum wheat bran extracts from the three varieties are shown in **Table 2.**

Table 2: Concentration of phenolic compounds in durum wheat bran extracts

	TPC*	TFC**	CTC**
Chen's	1.817±0.231	0.594±0.116	1.345±0.850
Siméto	1.678±0.051	0.543±0.102	1.526±0.193
Vitron	1.813±0.075	0.503±0.077	1.587±0.182

*mg AGE/g DWB, **mg CE/g DWB

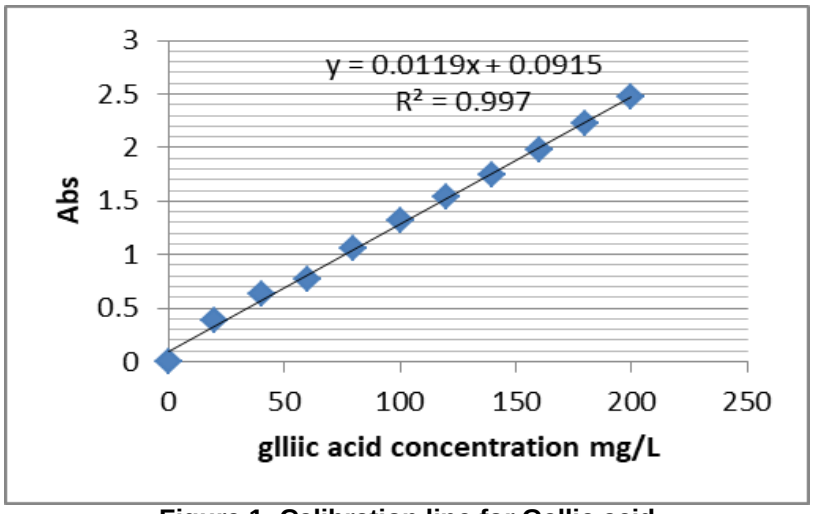

Figure 1: Calibration line for Gallic acid

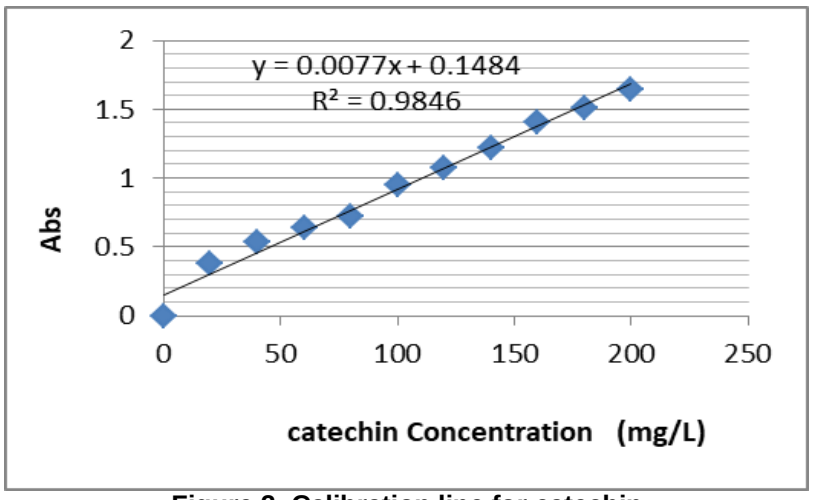

Figure 2: Calibration line for catechin

The total phenol contents for Siméto, chen's and vitron varieties are 1.678±0.046 mg GAE/g of DWB, 1.817±0.21 mg GAE/g of DWB and 1.813±0.068 mg GAE/g of DWB respectively. The flavonoid concentration is 0.543±0.091mg CE/g DWB (Siméto), 0.594±0.10 mg CE/g DWB (Chen's) and 0.503±0.069 mg CE/g DWB (Vitron). The condensed tannin content is 1.526±0.173 mg CE/g DWB for Siméto, 1.345±0.176 mg CE/g DWB for Chen's and 1.587±0.163 mg CE/g DWB for Vitron. No significant differences were recorded between the concentrations of the three phenolic compounds in the varieties studied. Total phenols (p=0.259), flavonoids (p=0.382) and condensed tannins (p=0.748).

Scavenger assay of DPPH radical

The results of DPPH radical scavenging activity (IC50) for the extracts of durum wheat bran of the three varieties and the different reference standards (ascorbic acid, gallic acid) are shown in Table 3.

The extracts of different varieties of DWB have low antioxidant activity compared to standards, with an IC50 of 35.16±0.15 mg/ml, 33.50±0.22 mg/ml and 40.79±0.71 mg/ml respectively for Chen's, Siméto and Vitron.

Table 3: IC50 values for DWB extracts and reference standards.

Extracts	IC50 (mg/ml)
Chen's	35.16±0.15
Siméto	33.50±0.22
Vitron	40.79±0.71
Ascorbic Acid	0.087±0.02
Gallic Acid	0.095±0.01

FRAP assay

The antioxidant power of DWB extracts was evaluated by FRAP assay in **Table 4**

Table 4: Fe++ concentrations in mmol/g of extracts

Extracts	antioxidant power *
Chen's	0.62±0.013**
Vitron	0.54±0.059**
Siméto	0.71±0.022**
Catéchine	4.81±0.004
Gallic Acid	4.62±0.081
Ascorbic Acid	4.38±0.009

*Mmol Fe++/G of extract, **p=0.004

The results show a significant difference (p=0.004) between the extracts of the different varieties, with 0.62±0.01 mmol Fe++/g for the Chen's variety, 0.54±0.05 mmol Fe++/g for the Vitron and a concentration of 0.71±0.02 mmol Fe++/g for Siméto variety. The reducing capacity of the extracts is less important compared to the standards used (catechin, gallic acid and ascorbic acid) with 4.81 mmol Fe++/g, 4.62 mmol Fe++/g and 4.38 mmol Fe++/g respectively.

β-carotene bleaching method

According to the bleaching kinetics of β-carotene **(Figure 3)**, no significant difference was found among the three DWB extracts of Chen's, Vitron and Siméto with p=0.893, and the percentages of inhibitory activity are 30.4±1.8%, 35.4±0.00% and 39±1.6% respectively **(Table 5)**. According to the bleaching kinetics of β-carotene, no significant difference observed (p=0.189) between the percentage's inhibition of the standards catechin (29.3±1.2%) and gallic acid (25.8±1.7%) and that of ascorbic acid (52.2±2.2%). The results of this assay show that the extracts of DWB having a high antioxidant activity compared to the reference standards. According to the percentage of inhibition, the Antioxidant Activity of the extracts is in the following order: Ascorbic acid> Siméto> Vitron> Chen's> Catechin> Gallic acid.

Table 5: Percentages of antioxidant activity of durum wheat bran extracts

Extracts	% of activity
Chen's	30.4±1.8*
Vitron	35.4±0.00*
Siméto	39.0±1.6*
Catechin	29.3±1.2
gallic acid	25.8±1.7
ascorbic acid	52.2±2.2

*p=0.133

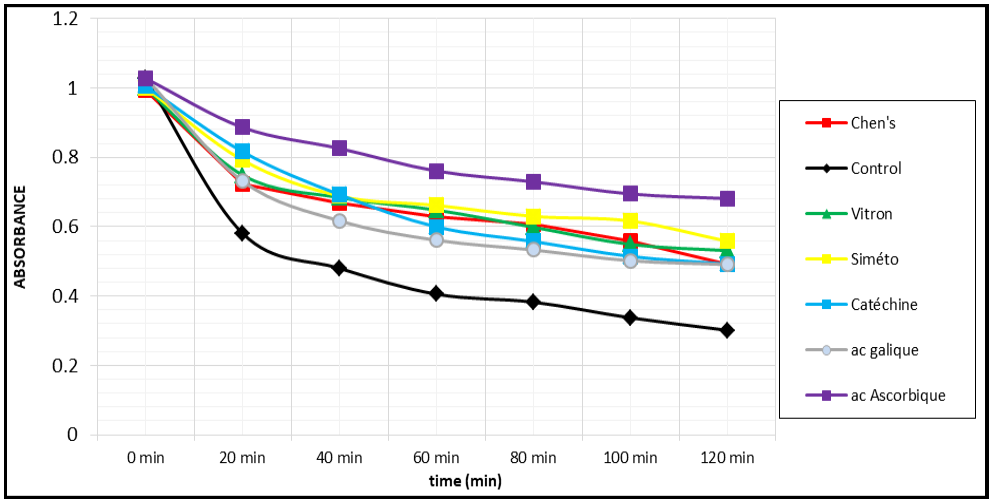

Figure 3: Bleaching kinetics of β-carotene against time for the three DWB varieties extract and reference standards

Chelating effect of ferrous ions (FIC)

The analysis of the ferrous ion chelating activity of our extracts shows a strong FIC activity. No significant difference (p=0.133) for the chelating activity observed between the three DWB extracts of the varieties, and the difference is no significant according to the concentrations of the extracts for this test with p=0.225. At the highest concentration (5 mg/ml), DWB extracts showed the highest FIC activity with 98.14%, 97.46% and 95.80% for Chen's, Vitron and Siméto respectively.

The extracts revealed a chelating effect strongly superior to the reference standards used catechin, gallic acid and ascorbic acid except for EDTA which gives an activity of 98.19% almost the same activity of our extracts at the highest concentration **(Table 6)**.

Table 6: Percentage (%) chelation of ferric ions in DWB extracts and standards

Extracts	1.75 mg/ml	2.50 mg/ml	5.00 mg/ml
Chen's	96.58±0.97	97.26±2.86	98.14±1.11
Vitron	94.72±1.33	95.70±1.18	97.46±0.97
Siméto	77.91±0.29	89.44±0.56	95.80±0.83
EDTA	97.86±0.08	98.10±0.08	98.19±0.14
Catechin	31.66±0.70	37.51±1.04	41.69±0.29
Ascorbic acid	29.11±1.41	30.13±0.35	32.08±1.26
Gallic acid	28.27±0.14	30.50±0.28	34.08±2.12

Phenolic compound contents

Phenolic acids and flavonoids represent the most common form of phenolic compounds found in whole wheat where they are among the major and most complex groups of phytochemicals[27]. It is well known that phenolic compounds are concentrated in the bran and germ fractions of wheat that are removed during the milling of wheat into white flour[12]. It is difficult to compare the results obtained in this study to those of previous studies because of the range of environmental factors and durum wheat cultivars used, varying testing methods, extraction solvent, differences in the extraction protocol and units of measure[28]. The results obtained in table 1 for total phenol concentrations are similar to those found by Vaher et *al*., 2010[12] who reported that wheat bran layers have the highest total phenol content, ranging from 1.258 to 3.157 mg GAE/g. However, Abozed et *al*., 2010[29] also found high concentrations of total phenol: 1.99 mg GAE/g for common wheat bran and 2.64 mg GAE/g for DWB with 70% hydro-ethanol extraction. Povilaitis et *al*., 2015[11] found a total phenol concentration between 1.58 and 1.78 mg AGE/g of wheat bran, they showed that variations in the concentration of total phenols between different particle size fractions for the same type of bran were not remarkable. The studies of Zhu et *al*., 2010[30] found a concentration close to our results which are 1.24±0.026 mg AGE/g of bran. In comparison with other works, our results confirm that the DWB has a considerable concentration in TPC. On the other hand, Flavonoids are an important class of phytochemicals in wheat, contributing to the health beneficial properties[31].

Our results show a low concentration in the bran extracts with an average between 0.503 and 0.594 mg CE/g of DWB because they are found largely in wheat germ and relatively low concentrations in bran[6] and the higher content of flavonoids existed in whole wheat than bran[29]. For flavonoid results, our concentrations are higher compared to other results. In a previous study by Abozed et al., 2010[29], showed that flavonoid concentrations are 223.96 µg CE/g for common WB and 258.04 µg CE/g for DWB. The results of Brewer et *al*., 2014[32] revealed that the level of flavonoids in wheat bran at different particle sizes ranged from 177.05 to 206.74 µg CE/g, and the results of the study indicate that flavonoids are concentrated in the bran. The mean total flavonoids were 213.04 and 259.31 mg CE/kg in bran and durum wheat bread, respectively. Further results from Ziliç et *al*., 2012[27] indicate that flavonoids are concentrated in the bran, and the mean total flavonoid content was 259.31 mg CE/kg in DWB. For condensed tannins content (CTC) results, we found it very difficult to compare these results with another research. We are fortunate to be the first to determine their concentration in DWB in such a study.

Previous work Mentioned that the variation of phenolic compound concentration in durum wheat bran affected by the geographic location, climatic condition and genotypes of varieties. Moreover, other studies suggested that the biotic conditions (species, organ and physiological stage) and abiotic stresses (salinity, luminosity, water deficit and edaphic factors) may enhance the phenolic metabolism as a response to oxidative stress[20]. However, Moore et *al*., 2006[33] motioned that the Environment and genotype are also important factors. Although in our results no significant variation in phenolic compounds observed among the DWB of the three varieties. The values obtained in our studies appear to be higher than those reported by Brandolini et *al*., 2013[34], Žilić et *al*., 2013[31] in literature for different durum wheat genotypes and/or agro-climatic areas. The presence of antioxidant compounds and the level of antioxidant activity in durum wheat are significantly affected by the genotype, the growing area[35]**,** and environmental conditions[31].Further study is needed to explore how these parameters change across environments, as genotype by environment interactions may have a high implication on the antioxidative capacity in plants. Although a relatively small number of genotypes was studied, high variability for most phenolic compounds and the antioxidant capacity was obtained in species[31]. It is known that the antioxidant properties of the wheat grain are significantly influenced by the genotype and environmental conditions and that phenolic compounds may significantly contribute to the overall antioxidant capacity of wheat grains[31], it would be useful to determine more precisely which individual environmental factors contribute most to this variance[33].

The antioxidant activity

The most harmful free radicals are hydroxyl ($HO^{\cdot}$) and hydrogen radicals produced in ionizing radiation or environmental toxicology reactions, as well as superoxide radicals (O_2^{-}) which are produced in mitochondrial electron transport reactions. Naturally, superoxide dismutase rapidly forms hydrogen peroxide (H_2O_2) from (O_2^{-}). Free ferrous iron (Fe^{2+}) and copper (Cu^{+}) participate in Fenton reactions with H2O2 to produce hydroxyl radicals and hydroxide anions, oxidizing the metals to Fe^{3+} and Cu^{2+}. The oxidized free iron or copper can then oxidize the H^2O^2 to form hydroperoxyl radicals ($HOO^{\cdot}$) and protons (H^{+}), reducing the metals to Fe^{2+} and Cu^{+}, so that new peroxidation reactions can occur cyclically and this leads to a lot of cellular, enzymatic, vascular dysfunctions[36]. The bran is responsible for the antioxidant capacity of wheat as it is rich in phenolic compounds. About 95% of phenol compounds are bound to cell wall polysaccharides and they are altogether indicated as dietary fiber-phenolic compounds[37]. In common with many other phytochemicals, phenolic acids and flavonoids were mostly in insoluble forms in the wheat bran layer ester-linked with cell wall components such as arabinoxylans and lignin. On the other hand, only a small percentage of soluble low molecular weight antioxidants (about 6%), which are free from chemical or physical interaction with other macromolecules, are present in wheat bran[38].

The results of the antioxidant activity studied using a variety of in vitro methods including DPPH radical scavenging activity, bleaching of β-carotene, FRAP reducing power and FIC chelation activity, showed considerable antioxidant potential, which may be partly responsible for the medicinal properties of durum wheat bran. The antiradical activity of the DPPH and FRAP test was found to be quite low compared to the other methods. Our FIC test results and the β-carotene bleaching test are very important. While all the assays presented in this paper have been evaluated and utilized by researchers to investigate antioxidant properties. The assay results for phenolic compounds and the antioxidant activity of DWB extracts show low positive and negative correlation except between the antioxidant activity of β-carotene test with CTC which is positively correlated with r^2=0.799 and the antioxidant activity of the FIC test with TPC which has a perfect positive correlation with r^2=0.966, and

we note that the correlation between TFC and DPPH scavenging activity is positively remarkable with r^2=0.780, because the most of the flavonoids possess strong antioxidant properties following chain-breaking mechanism and they can act as antioxidants by acting as hydrogen donors or by acting as chelating agents and may help to control the extent of lipid peroxidation by chelating copper ions[39]. Žilić et *al*., 2013[31] find that the total antioxidant capacity had a positive correlation with the free total phenolics in bread wheat and durum wheat (r^2=0.76 and 0.80). However, considering that most of the phenolic compounds in cereals are bound to the insoluble polysaccharide, the antioxidant capacity of wheat is mostly dependent on this phenolic form. A significant correlation between antioxidant properties for wheat grain and fractions have been previously reported in numerous studies, others found significant correlations between DPPH scavenging capacity and TPC[33]. So TPC is reported to be directly associated with antioxidant activity[39]. Liyana-Pathirana and Chahidi in 2007[40] reported that only the bran fraction showed higher antioxidant activity than the other fractions obtained after wheat milling and found that the ability to scavenge DPPH radicals in wheat fractions was in the order of bran> shorts> feed flour> whole grain> flour, for wheat cultivars.

These results were supported by those of Higuchi in 2014[8], Onyeneho and Hettiarachchy in 1992[41], who finds that Wheat bran has a higher antioxidant activity than other milled fractions and the extract of wheat bran having a high concentration of phenolic acids, were shown to have stronger antioxidant activity than other fractions of wheat. Phenolic compounds are considered to be free radical scavengers, and their antioxidant properties depend on their chemical structure. Specifically, these properties depend on their ability to donate hydrogen or electron and their ability to delocalize the unpaired electron within the aromatic structure[42]. The results obtained show that the antioxidants contained in DWB extracts can trap free radicals and reduce oxidants. The electron donor properties of wheat extract thereby neutralizing free radicals by forming stable products. The outcome of the reducing reaction is to terminate the radical chain reactions that may otherwise be very damaging[38].

The DWB examined in this study demonstrated good reducing capacity thereby acting as efficient reductones. This is in agreement with Liangli, 2007[43] who reported that phenolic acids, which are covalently bound to the insoluble wheat bran matrix, possess different antioxidant functions. Among various species of transition metals, iron is known as the most powerful pro-oxidant due to its high reactivity[44], and the Chelating agents can trap or deactivate metal ions and potentially inhibit the transition metal-mediated oxidative processes. The wheat bran has a substantial capacity for binding metal ions and the major active components have been proposed as dietary fibers or phytate in bran[45]. The work of Wang et *al*., 2013[46] indicated that the metal-chelating capacity of phenolic compounds does not seem to be related to their effectiveness in reducing power and radical scavenging activities. Wheat bran has the strongest activity bonds for the reducing power and chelating activity of ferrous ions[30].

The β-carotene test is a complementary technique that simulates the lipid peroxidation reaction in vitro. It consists of measuring the bleaching of β-carotene resulting from its oxidation by the degradation products of linoleic acid. The oxidation of the latter is catalyzed by heat (50°C)[47]. The addition of extracts of DWB and standards has the effect of delaying the spread of the reaction. The activity of our extracts was moderately high compared to the reference molecules. Comparison with other work is a delicate task because of certain limitations of the technique, such as the different concentrations of extracts used by the researchers and also the thermally induced oxidation of linoleic acid which is not controlled, which often induces variability in the data[47]. Finally, the extracts give a strong activity due to their richness in cell cycle inhibiting compounds, antioxidant capacity and chelation of metals such as phenolic acids, Alkyresorcinols and phytic acid[6]. These results indicated that wheat bran may replace synthetic antioxidants in food formulations and play a major role in human health[29].

Conclusion

The results of our study showed that durum wheat bran is a by-product rich in phenolic compounds and has considerable antioxidant activity. This antioxidant power testifies the presence of some active principles that can be valorized in the pharmaceutical industry for the prevention and therapy of diseases due to oxidative stress. The establishment of a diet rich in wheat bran as a replacement for synthetic antioxidants in food formulations can play an important role in human health and present undoubtedly a therapeutic and dietetic interest.

Conflict of interest statement
The authors declare that there is no conflict of interest.

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