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Genotype impact on antioxidant potential of hull and kernel in Persian walnut (*Juglans regia* L.)

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Abstract

The objective of this work was to investigate relative antioxidant performance of seven different walnut (*Juglans regia* L.) genotypes including KZ7, KZ9, KZ15, OR126, Sebin, Pedro and Chandler grown on the Agricultural Research Center of West Azerbijan province, Urmia, Iran. Phenolics, flavonoids and antiradical capacities in hull and kernel were analyzed. The kernel of Pedro (66.55±4.98 mg/g of GAE) and hull of Sebin (122.26±1.34 mg/g of GAE) showed the highest value for phenolic compounds. The highest flavonoid compound was obtained in the kernel of KZ7 (13.57±2.27 mg/g of CEs) and hull of the Sebin (49 ±3.17 mg/g of CEs). Phenolics analysis was performed by High performance liquid Chromatography (HPLC) and 10 compounds were identified and quantified including ascorbic acid, gallic acid, rutin, caffeic acid, p-hydroxy benzoic acid, vanillic acid, p-cumaric acid, syringic acid, ferulic acid and sinapic acid. In hull extracts, the highest phenolic contents was found in KZ9 (gallic acid), KZ7 (rutin and sinapic acid) and KZ15 (p-hydroxy benzoic acid) while in kernel extracts, KZ9 (syringic and gallic acids), Chandler (sinapic acid) and KZ7 (p-cumaric and ascorbic acids). On the basis of the presented results, Iranian walnut genotypes could be considered as potential sources of natural antioxidants.

Introduction

The *Juglans* genus (Juglandaceae) comprises several species and is widely distributed throughout the world. The plant is found in the temperate areas and cultivated in the United States, western South America, southern and eastern Asia, and central and southern Europe (Oliveira *et al*., 2008). Persian walnut (*Juglans regia* L.) is one of the most economically important cultivated species for its timber and nutritious nuts (Mahmoodi *et al*., 2013). It also has medicinal importance for human health (Rahimipanah *et al*., 2010) and used as a traditional remedy for treating cough, stomach ache and cancer in Asia and Europe (Kamyab *et al*., 2010).

Free radicals are responsible for oxidative deterioration, health damage and accelerated aging (Cheng *et al*., 2014). Antioxidants are able to neutralize free radicals and decreasing the risk of degenerative diseases by reduction of oxidative stress and inhibition of macromolecular oxidation (Lobo *et al*., 2010). They have a potential role in preventing cardiovascular disease, some neurological disorders or certain inflammatory processes (Popa *et al*., 2011). However, utilization of natural sources antioxidants such as fruits and vegetables has been intensified due to the potential toxicity of synthetic antioxidants (Ahmad *et al*., 2015). Walnuts and peanuts are known to have significant antioxidant contents (Amarowicz *et al*., 2005; Blomhoff *et al*., 2006; Isanga and Zhang, 2007; Miraliakbari and Shahidi, 2008). Among the nuts, walnut polyphenols have been shown to perform the highest lipoprotein-bound antioxidant activity. The descending order of decreasing efficacy for raw nuts has been reported as walnut > cashew > hazelnut ~ pecan ~ almond ~ Macademia > pistachio > Brazil > peanut (Vinson and Cai 2012). Green walnuts, shells, kernels and seeds, bark, and leaves have been also used in the pharmaceutical and cosmetic industries (Sharafati Chaleshtori *et al*., 2011).

The main objective of the present work was to determine phenolic and flavonoid contents as well as to evaluate antioxidant capacity of hull and kernel in different walnut genotypes (KZ15, KZ9, KZ7, OR126, Chandler, Pedro and Sebin) grown in the same location in Iran.

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Materials and Methods

Chemicals

All chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Sample preparation

Genotypes were selected from Kahriz (KZ7, KZ9, and KZ15) and Urmia (OR 126). The Sebin (Turkey), Pedro (France) and Chandler (USA) were picked up as foreign cultivars for comparison. All genotypes were collected from Agricultural Research Center in Urmia. Each Sample was randomly collected from fresh leaf tissues of at least 3 individual plants in spring of 2013. Three samples were considered for each genotype. Tissues were frozen in liquid nitrogen and stored at -80°C prior to analysis.

Methanolic extraction

The hulls and kernels were ground to fine powder. An extraction using pure methanol (in soxhlet apparatus at 60°C for 30 min) was applied to obtained powder. The samples were centrifuged for 20 min at 4000 X g. The supernatant was filtered through filter paper and stored at 4°C until analysis for one week.

Determination of total phenolic content (TPC) and total flavonoid content (TFC)

The concentrations of phenolic compounds in all samples were expressed as mg gallic acid equivalents (GAEs), determined with Folin–Ciocalteu Reagent (FCR) according to the method of Slinkard and Singleton, 1997 with minor modifications. The TFC of the all extracts was quantified using a modified colorimetric method (Yang *et al*., 2009).

Determination of 2, 2-diphenyl-1-picrylhydrazyl (DPPH), superoxide, nitricoxide and hydrogen peroxide radical scavenging activity

DPPH radical scavenging activity was determined as described by Wu *et al*. (2003) with slight modification. For O2- assay, the superoxide anion radicals were generated by a pyrogallol autoxidation system (Jing and Zhao, 1995). NO inhibition was estimated by the use of GriessIllosvoy reaction (Garrat, 1964). In this investigation, GriessIllosvoy reagent was modified by using naphthyl ethylenediamine dihydrochloride (0.1% w/v) instead of 1-napthylamine (5%). The Hydrogen peroxide-scavenging activity of extract was determined by the method of Ruch *et al*. (1989).

Extraction and hydrolysis for HPLC

For HPLC analysis, 500 mg of dried and powdered plant material was submitted to an extraction with 50% methanol/water for 2 h at room temperature. The plant extract was hydrolyzed with 1.2 M HCl by refluxing in a water bath for 1 h. All samples were filtered through a 0.45 µm pore size syringe-driven filter before injection (Hertog *et al*., 1992).

Chromatographic conditions

Phenolic compounds of the extracts were analyzed by HPLC KNAUER system (Germany) equipped with UV–Vis detector and an Eurospher 100-5 C18 column (25 cm X 4.6 mm; 5 µm). The mobile phase consisted of purified water with 2% acetic acid (A) and acetonitrile (B). Solvent gradient was used as follows: 0 - 5 min isocratic at 85% A, 5 - 19 min (14 min) a linear gradient of 85% A to 100% B. After termination of the cycle, 15 min of column equilibration (85% A) was allowed prior to next injection. The column temperature was set at 25°C. Chromatographic data were acquired and processed using Chrom Gate accompanying the HPLC equipment. The detection wavelength was set at 254 nm for ascorbic acid, gallic acid, rutin, caffeic acid, hydroxyl benzoic acid, vanillic acid, p-cumaric acid, syringic acid, ferulic acid and sinapic acid. The temperature was set at 25°C and the flow rate at 0.5 ml / min. Typical HPLC chromatogram of the walnut hull extract recorded at 254 nm (Figure 1).

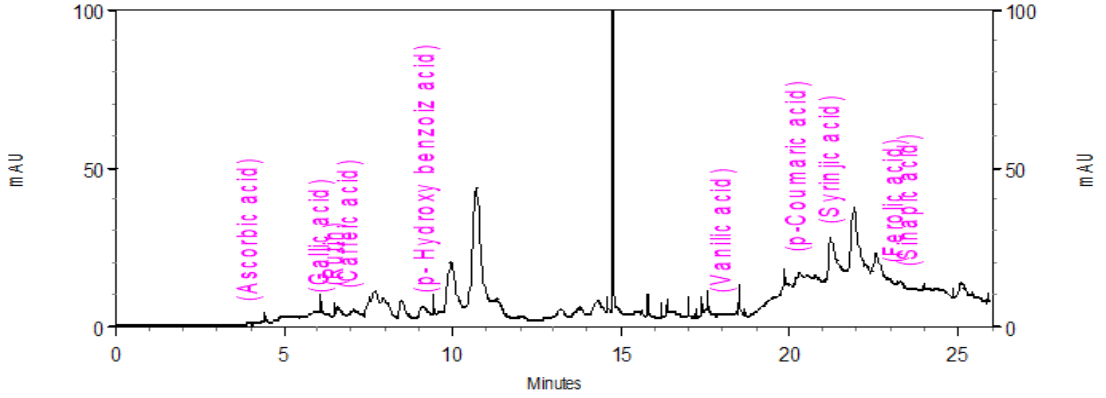

Figure 1. Typical HPLC chromatogram of the walnut hull recorded at 254 nm

Table 1.Total phenolic [gallic acid equivalents (GAEs)] and flavonoid contents [catechin equivalents (CEs)] in seven walnut genotypes grown in West Azerbaijan of Iran

Genotypes	Hull	Kernel
Phenolic content (mg/g)		
KZ7	^{c}104.71±1.58	^{b}49.64±0.64
KZ9	^{bc}107.21±1.27	^{b}46.64±0.63
KZ15	^{c}100.53±3.07	^{b}49.42±0.63
OR126	^{c}99.98±6.25	^{a}64.00±1.91
Sebin	^{a}122.26±1.34	^{b}49.70±1.91
Pedro	^{c}107.49±5.25	^{a}66.55±4.98
Chandler	^{ab}115.29±8.32	^{b} 49.00±0.63
Flavonoid content (mg/g)		
KZ7	^{bc} 27.5 ±1.27	^{a}13.57±2.27
KZ9	^{bc}31.07±0.61	^{b}10.11±1.16
KZ15	^{bc}28.45±0.24	^{a}13.67±2.32
OR126	^{bc}31.01±0.23	^{ab}11.26±0.89
Sebin	^{a} 49.00±3.17	^{c} 5.86±0.91
Pedro	^{bc}31.53±1.65	^{c}4.82±1.80
Chandler	^{b} 16.71±0.38	^{c} 4.55±0.46

Data are means of three replicates with standard errors (Mean ± S.E, n = 3), $p < 0.05$. Values in the same column with different letters present significant differences $p < 0.05$

Statistical analysis

All the assays were carried out in triplicate. The results are expressed as mean values and standard error (SE) of the mean. Data analyses were performed using SPSS software version 16 and the means were compared using Tukey's multiple range test (TMRT) at $p < 0.05$ following analysis of variance (ANOVA).

Results and Discussion

Phenolics and flavonoids

Phenolic compounds contribute to the antioxidant activity of plant materials due to their scavenging ability (Djeridane *et al*., 2006). Foods rich in phenolic acids are considered as health beneficial. However, it's difficult to understand how much of that benefit is actually related to phenolic acids, or to other nutrients also found in those foods. Meanwhile, nutrition improvement and security of the food is highly affected by biodiversity which plays a remarkable role in developing agricultural practices and strategies against malnutrition (Toledoa and Burlingameb 2006).

The total phenol content in walnut green hull methanolic extracts has been presented in Table 1. No significant difference was observed between KZ7, KZ9, KZ15, OR126 and Pedro. The Sebin presented the major phenolics, with 122.26 mg/g of GAE, being 1.06–1.2 fold higher compared to the other genotypes. The lowest amount was obtained for OR126 with 99.98±6.25 mg/g of GAE (Table 1). No significant difference was observed between KZ7, KZ9, KZ15, Sebin and Chandler in kernel extracts. KZ9 genotype was the poorest in TPC in kernel (46.64±0.63 mg/g of GAE) and Pedro presented the highest value (66.55±4.98 mg/g of GAE). As expected, there were significant differences among hulls and Kernels. The results indicate that the hulls contain much higher phenolic compounds than are the kernels.

The data presented in Table 1 indicate that the highest flavonoid content of 49 ±3.17 mg CE/g was observed in the Sebin hull. Chandler variety was the poorest in flavonoids in both hull (16.71±0.38 mg CE/g) and kernel (4.55±0.46). The KZ15 and KZ7 presented the highest flavonoid value of kernel (13.67±2.32 and 13.57±2.27 mg CE/g), respectively. In accordance with the major TPC, the highest flavonoid content was observed in hull of Sebin genotype.

HPLC phenolic profile

In general, genotypes presented ten identified phenolic compounds (Table 4). Phenolic compounds in walnut hulls and kernels were as follow: ascorbic acid, gallic acid, rutin, caffeic acid, hydroxyl benzoic acid, vanillic acid, p-cumaric acid, syringic acid, ferulic acid and sinapic acid. Stampar *et al*. (2006) identified other phenolics due to the fact that they collected walnuts with green husk just before the hardening of the endocarp.

The individual phenolic compounds were identified by comparing their UV–vis spectra with those obtained from standards. In our study,

Table 4. phenolic profile (mg/100 g) of walnut hull and kernel in seven different genotypes. Values represent mean

Phenolics	Ascorbic acid	Gallic acid	Rutin	Caffeic acid	P-hydroxy benzoic acid	Vanillic acid	p-coumaric acid	Syringic acid	Ferulic acid	Sinapic acid
Hull										
KZ7	-	3.86	7.17	2.83	1.59	0.51	0.85	14.54	1.81	77.13
KZ9	0.07	4.53	-	1.54	1.59	0.12	1.37	-	1.49	0.13
KZ15	0.07	3.35	1.38	1.50	2.38	0.10	1.64	-	-	0.36
OR126	0.07	-	-	1.58	1.75	0.11	-	1.56	1.48	0.03
Sebin	0.07	3.52	3.05	1.45	1.58	0.12	-	1.31	1.76	0.20
Pedro	0.11	2.85	5.42	1.69	1.58	0.11	0.76	-	-	5.39
Chandler	-	2.10	3.08	-	1.64	0.10	2.30	1.23	1.48	7.95
Kernel										
KZ7	5.20	1.02	1.65	1.89	0.20	2.11	-	1.15	6.33	5.20
KZ9	-	8.19	1.27	1.85	1.80	0.14	1.69	85.06	1.64	0.45
KZ15	0.07	2.17	1.75	1.57	1.58	0.10	0.98	7.89	1.56	4.70
OR126	0.15	3.40	-	1.56	3.35	0.09	-	6.21	1.48	9.05
Sebin	-	4.15	1.81	1.49	-	0.09	-	1.46	1.55	0.20
Pedro	3.25	1.36	1.43	2.14	0.06	-	5.63	1.62	3.02	3.25
Chandler	-	7.39	2.75	1.57	2.65	0.13	3.04	8.16	1.51	12.82

(-) implies absence

variations in phenolics contents were observed in hulls of genotypes as well as kernels (Table 4). In hull extracts, the highest phenolics were found in KZ9 (gallic acid), KZ7 (rutin and sinapic acid) and KZ15 (p-hydroxy benzoic acid).

But in kernel extracts, among the identified phenolic compounds, the highest phenolics were found in KZ9 (gallic and syringic acids), Chandler (sinapic acid) and KZ7 (p-cumaric and ascorbic acids). The presence of ascorbic acid was not detected in Chandler, Sebin and KZ9 kernel extract. Results of HPLC confirm the antiradical activities. In this study, a direct relationship between the ability of radical collecting, with the amount of phenolic compound was not observed. Our result is in accordance with previously published data (Akbari *et al*., 2012) reporting no direct correlation between phenol content and antiradical activity due to reduced activation of collecting. Colaric *et al*. (2005) have also reported the presence of several polyphenols including chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, sinapic acid, ellagic acid and syringic acid as well as syringaldehyde (hydroxybenzoic aldehyde) and juglone (quinone) in walnut kernels.

Another study conducted by Zhang *et al*. (2009) identified the presence of pyrogallol, p-hydroxybenzoic acid, vanillic acid, ethyl gallate, protocatechuic acid, gallic acid and 3,4,8,9,10-en tahydroxydibenzo[b,d]pyran-6-one in kernels of J.regia. In our analysis, different genotypes and even different sections of plant contained different phenolic compounds. These compounds are important constituents of plants and are known for their potent antioxidant activity. Gallic, p-coumaric, ferulic, Vanillic, caffeic and syringic acids have been reported to reduce and decolorize DPPH radicals by their hydrogen donating ability (Karamać *et al*., 2005).

DPPH radical scavenging capacity

In this study, the antiradical capacity against ROS species was accessed by scavenging activity on DPPH radicals. All the studied hull extracts exhibited high scavenging properties against DPPH radicals, varying from 76.71% (OR126) to 89.81% (Chandler). The extracts obtained from kernel of Pedro showed the highest antioxidant activity, scavenging 27.48% of the free DPPH radicals while KZ15 presented the lowest activity with scavenging effect of 15.27%.

DPPH is a stable free radical with purple color. DPPH assay has been widely used to determine the free radical scavenging activity of plants (Oliveira *et al*., 2008). Antioxidant molecules scavenge the free radicals by hydrogen donation which consequently changes the purple color to light yellow resulting in a decrease in absorbance at 517 nm. Free radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation (Ferreres *et al*., 2007).

A significantly poor linear correlation was found between the total phenols content and DPPH scavenging capacity of hull (R^2=0.119) (Table 3). The hull extracts showed higher DPPH scavenging

Table 2. DPPH, superoxide, nitric oxide and H_2O_2 radical scavenging (%) in seven genotypes of *Juglans regia* L. hulls and kernels grown in West Azerbaijan of Iran

Genotypes	DPPH scavenging (%)	Superoxide scavenging (%)	Nitric oxide scavenging (%)	H_2O_2 scavenging (%)
Hull				
KZ7	^{c}83.82±0.85	^{b}52.52±4.25	^{a}31.35±1.70	^{c}197.82±2.74
KZ9	^{c}83.66±1.22	^{b}50.90 ±4.98	^{c}20.63±1.27	^{c}184.00±3.20
KZ15	^{b}87.15±0.4	^{cd}31.31 ±4.60	^{cd}25.40±1.35	^{d}189.70±3.54
OR126	^{d}76.71±0.67	^{b}52.17 ±1.68	^{d}24.14±125	^{c}193.92±2.60
Sebin	^{c}83.45 ±0.92	^{b}58.68±6.35	^{ab}30.81±3.18	^{a}236.86±3.40
Pedro	^{b}86.40 ±1.59	^{a}97.00±7.00	^{bc}28.10±1.00	^{f}179.96± 4.20
Chandler	^{a}89.81 ±1.21	^{a}98.48±7.20	^{ab}29.00±1.48	^{b}202.49±5.10
Kernel				
KZ7	^{d}17.06±0.38	^{b}84.04±4.20	^{b}40.81±3.11	^{b}236.67±1.82
KZ9	^{c}16.85±0.34	^{a}98.00±4.20	^{a}54.68±1.56	^{d}229.56±3.00
KZ15	^{e}15.27±0.32	^{ab}90.00±3.00	^{b}42.16±1.64	^{a}239.80±2.00
OR126	^{d}17.06±0.38	^{ab}91.00±2.9	^{c}35.76±1.33	^{e}223.16±1.17
Sebin	^{c}16.85±0.34	^{ab}90.00±3.2	^{a}53.60 ±3.42	^{ef}221.95±3.40
Pedro	^{a}27.48±1.01	^{ab}91.00±3.1	^{a}53.33±1.00	^{f}219.84± 4.20
Chandler	^{b}25.00±1.21	^{a}98.48±4.2	^{d}18.73±2.90	^{c}233.53±3.66

Data are means of three replicates with standard errors (Mean ± S.E, n = 3), $p < 0.05$. Values in the same column with different letters present significant differences $p < 0.05$

Table 3. Correlation coefficient (R^2) between bioactivities and total phenolic and flavonoid contents

	Correlation coefficient (R^2)			
	Phenol		Flavonoid	
	Hull	Kernel	Hull	Kerne
DPPH	0.119	0.078	0.17	0.595
Superoxide	0.201	0.028	0.097	0.244
Nitric oxide	0.245	0.008	0.017	0.002
Hydrogen peroxide	0.595	0.015	0.597	0.604

capacity than kernel extracts. Low correlation coefficient was also observed between phenolic content and antiradical activity in kernel extracts (R^2=0.078). The poor relation between phenolics and DPPH in hull and kernel could be due to, the major antioxidant components might not be phenolics and could be other food antioxidants present such as sterols, tocopherols, carotenoids, chlorophylls, amino acids, proteins and vitamin E (Shahidi 2000). However, the strong and positive correlation was detected between DPPH radical scavenging activity and p-coumaric acid of Hull (R^2=0.64) and kernel (R^2=0.82).

The correlation between flavonoid content and DPPH was weakly related in hull (R^2=0.17), while positively and significantly associated in kernel (R^2=0.595). Flavonoids are not always phenolic compounds, depending on position of OH in flavonoid. Flavonoid with OH in A ring and or B ring is called as phenolic. However, the OH with ortho position in C3'-C4', OH in C3, oxo function in C4, double bond at C2 and C3 have the highest influence on antioxidant activity of flavonoid (Heim *et al*., 2002) . Hence, it is predicted that flavonoids in hull of Sebin (49 .00 mg/g of CEs) and kernel of KZ7, KZ9, KZ15 and OR126 were flavonoids with no OH in ortho C3', 4', OH in C3, oxo function in C4, double bond at C2 and C3.

Superoxide radical scavenging capacity

No significant difference was observed between Pedro and Chandler compared to other genotypes in the inhibition effects of walnut hull extracts on the autoxidation of pyrogallol (Table 2).The O_2- scavenging activity values ranged from 31.31% to 99.29%. The Chandler exhibited highest activity for scavenging superoxide anion radical. No significant difference was observed between KZ7, KZ9, OR126 and Sebin. The kernel extracts also differed in their superoxide anion (O2-) radical scavenging activities. The O_2- scavenging activity values ranged from

84.04% (KZ7) to 98.48% (Chandler). The kernel extract was found to be more potent than hull extract. No significant difference was observed between KZ15, OR126, Sebin and Pedro. Chandler presented the highest O_2- radical scavenging activity in both hull and kernel tissues. Reactive free radicals, such as O_2- and peroxy radical (ROO.), are extremely reactive and are known to be a biological product in reducing molecular oxygen (Williams and Jeffrey, 2000). A weak correlation was detected between the total superoxide scavenging capacity with total phenolic (R^2 = 0.201 for hull, R^2= 0.028 for kernel) and flavonoid (R^2 = 0.097 for hull, R^2 = 0.224 for kernel) contents of the extracts (Table 3). It was proven that superoxide scavenging activity does not always correlate with the presence of large quantities of phenolics (Bozin *et al*., 2008). Therefore, the results suggest the presence of phenolic compounds with different capacity for scavenging in genotypes under this study. In our analysis, the hull of KZ7 (14.54 mg/100g) and kernel of KZ9 (85.06 mg/100g) contained the highest syringic acid content. Syringic acid has been shown to possess the strongest O_2-scavenging activity followed by ferulic acid (Zhou *et al*., 2006). In accordance to previous findings, superoxide radical scavenging was in association with gallic (R^2 = 0.81), syringic (R^2=0.49), sinapic (R^2 = 0.45), vanillic (R^2 = 0.42) and ferulic (R^2= 0.35) acids in kernel tissues.

Nitric oxide radical scavenging capacity

The nitric oxide scavenging ability of the methanolic walnut extracts was also analyzed. The study of the effect of extracts against this radical was a novelty point in this work. Nitric oxide (NO) is an abundant reactive species which acts as a signaling molecule in a large variety of physiological processes, including blood pressure regulation, smooth muscle relaxation, neurotransmission, defense mechanisms, and immune regulation. Generation of reactive nitrogen species alters the structure of proteins and inhibits their normal function (Valko *et al*., 2007).

In our study, NO was generated from sodium nitroprusside (SNP) and was measured by the Griess reagent. At physiological pH, SNP spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be estimated by the use of Griess reagent. Scavengers of NO compete with oxygen to reduce the production of NO. The maximum (31.35%) and minimum (20.63%) nitric oxide radical inhibition was achieved for KZ7 and KZ9 hull extracts, respectively. The KZ9 kernel presented the best property for No scavenging activity (54.68%) followed by Sebin and Pedro (53%) whereas Chandler showed the lowest activity (18.73%). In hull, NO radical scavenging capacity was lower compared to DPPH radical scavenging activity. The kernel extract was found to show better inhibition activity compared to hull extract. On NO assay, values obtained for walnut hull extracts varied from 18.73% to 54.68% in order of KZ9 < OR126< KZ15< Pedro<Chandler< Sebin < KZ7 (Table 2). Poor linear correlations were found between total phenolic and flavonoid contents with nitric oxide scavenging activity in hull (R^2=0.245 for phenolic, R^2=0.017 for flavonoid) and kernel (R^2=0.008 for phenolic, R^2=0.002 for flavonoid) (Table 3). Based on our correlation analysis, nitric oxide scavenging activity was associated with rutin (R^2=0.67) in hull and p- hydroxyl benzoic (R^2=0.40) and sinapic (R^2=0.93) acids in kernel.

H_2O_2 radical scavenging capacity

The extracts were also examined for their ability to act as OH radical scavenging agent. Radical scavenging activity of hydrogen peroxide in hull illustrates that the highest level (236.867%) was observed in Sebin and Pedro (179.96%) was found to contain the lowest scavenging activity (Table 2). In kernel, the highest level of hydrogen peroxide activity (239.80%) was detected in the KZ15, while the lowest level was shown in the Pedro (219.84%). The Pedro hull and kernel extracts (Table 2), showed the lowest hydrogen peroxide activity. In respect to hydrogen peroxide radical scavenging activity, minimal difference was observed between genotypes (219-239%).Interestingly, the hull of Sebin (236.86%) and kernel of KZ7 (239.80%) revealed almost the same activity. H_2O_2 is poorly reactive at physiological concentrations and is toxic to cells at 10-100 µl levels. It can cross biological membranes rapidly to form cytotoxic hydroxyl radicals (Siriwardhana and Shahidi, 2002). Significant linear correlations were found between total phenols and flavonoid contents with hydrogen peroxide in hull (R^2=0.595 phenolic, R^2=0.597 flavonoid) (Table 3). In kernel, phenolics and H_2O_2 radical scavenging was poorly associated (R^2=0.015), while flavonoid content and H_2O_2 showed significant correlation (R^2=0.604). However, no significant correlation was found between H_2O_2 scavenging and any of phenolic components of neither hull nor kernel. Therefore, the observed strong correlation could be due to flavonoid components of extracts.

Conclusion

The kernel of Iranian genotypes possesses higher

levels of flavonoids compared to the French (Pedro) and USA (Chandler) cultivars. Iranian genotypes have almost equal superoxide, nitricoxide, and H_2O_2 scavenging activities of kernels compared to foreign genotypes. The existence of positive correlation between flavonoid content and DPPH scavenging exhibits great antioxidant capacity of kernel due to presence of flavonoid. However, due to lack of high association between phenolics and bioactivities, the major antioxidant capacities of hull and kernel could be attributed to sterols, tocopherols, carotenoids, chlorophylls, amino acids and vitamin E. Further investigations are required to clarify the relationship between bioactivities and other components of the hull and kernel.

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