IDENTIFICATION OF THE DIVERSITY OF CULTIVATED PLANTS AND THEIR WILD RELATIVES FOR SOLVING FUNDAMENTAL AND APPLIED PROBLEMS

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Exploring extraction techniques for bioactive compounds, and investigating antioxidant properties in Moroccan oat lines

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The article highlights the importance of antioxidants, especially dietary phenolic compounds, in promoting human health, and discusses the potential of cereals, such as oats, known for their richness in avenanthramides. The study aimed to optimize the method of extracting antioxidants and phenolic compounds from oat grains, and their quantitative assessment was carried out. The material for the study included oat cultivars of Moroccan origin and interspecific oat lines. The extraction of phenolic compounds with ethanol was found to be the most effective. The study explored total phenolics, flavonoids, carotenoids, and total antioxidant activity, revealing genetic diversity among oat lines. As a result, a significant correlation was established between the phenolic content and antioxidant activity in the studied Moroccan oat lines. It is noteworthy that lines F11-5 (*Avena sativa* × *A. magna*) and F10-3 (*A. sativa* × *A. murphyi*), along with cv. 'Zahri' (*A. sativa*) and accession P1-1 (*A. magna*), exhibited high antioxidant activity and the highest total phenolic content, thus attesting to their health benefits and revealing the richness of Moroccan oat lines and cultivars for food use.

Keywords: oat, interspecific lines, cultivars, extraction, phenolic compounds, flavonoids, carotenoids, antioxidant activity

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ИДЕНТИФИКАЦИЯ ГЕНЕТИЧЕСКОГО РАЗНООБРАЗИЯ КУЛЬТУРНЫХ РАСТЕНИЙ И ИХ ДИКИХ РОДИЧЕЙ ДЛЯ РЕШЕНИЯ ФУНДАМЕНТАЛЬНЫХ И ПРИКЛАДНЫХ ПРОБЛЕМ

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Оценка методов экстракции биологически активных соединений и исследование антиоксидантных свойств марокканских линий овса

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В статье рассматривается биохимический потенциал зерновых культур, таких как овес, оказывающих положительное воздействие на здоровье человека благодаря высокому содержанию характерных для овса авенантрамидов, фенольных соединений и других антиоксидантов. Целью исследования являлась оптимизация метода извлечения антиоксидантов и фенольных соединений из зерна овса, проведена их количественная оценка. Материалом для исследования послужили сорта марокканского происхождения и межвидовые линии овса. Установлено, что экстрагирование фенольных соединений этанолом является наиболее эффективным. Изучены общее содержание фенольных соединений, флавоноиды, каротиноиды и общая антиоксидантная активность, что позволило выявить генетическое разнообразие среди образцов овса. В результате установлена значительная корреляция между содержанием фенолов и антиоксидантной активностью у изучаемых линий марокканского овса. Примечательно, что линии F11-5 (*Avena sativa* × *A. magna*) и F10-3 (*A. sativa* × *A. murphyi*), наряду с сортом 'Zahri' (*A. sativa*) и образцом P1-1 (*A magna*), сочетают высокую антиоксидантную активность с максимальным общим содержанием фенолов, что подчеркивает пользу овса для здоровья человека и свидетельствует о богатстве марокканских линий и сортов овса для пищевого использования.

Ключевые слова: овес, межвидовые линии, сорта, экстракция, фенольные соединения, флавоноиды, каротиноиды, антиоксидантная активность

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Introduction

Dietary plants combine different chemical families and amounts of naturally occurring phytochemicals. Inter alia, they include antioxidants which are biosynthesized by plants as a part of their biological system in order to minimize the destructive potential of oxidation reactions and strengthen their defense system (Halliwell, 1999; Naczk, Shahidi, 2006). Over the past decade, antioxidants, especially dietary phenolics, have received tremendous attention among food scientists and nutritionists owing to their protective effects against wide pathologies that humankind is faced with. Dietary antioxidants may promote general human health as potential treatment against various maladies, including certain cancers, chronic inflammatory diseases, and aging (Lolayekar, Shanbhag, 2012). Recent studies have revealed that many such ailments are related to oxidative stress from reactive oxygen and nitrogen species (Tsao, 2010). Besides, phytochemicals, especially polyphenols, are the predominant contributors to the total antioxidant activities of natural compounds or foods, since they reduce the rate of oxidation through two main mechanisms: by acting as hydrogen atom donators or as metal ion chelators (Gülçin, 2012; Tsao, 2010). Thus, several recent reviews have summarized information on the extraction, identification and quantification of these compounds from various natural substances, such as dietary plants or agricultural and food products (Marc et al., 2004; Naczk, Shahidi, 2006). These compounds were intensely studied and discussed but their beneficial effects were often ascribed to the intake of fruits, legumes, and vegetables (Cao et al., 1998; Kaur, Kapoor, 2001). Keeping in mind the need to increase the consumption of bioactive dietary compounds, other natural food sources are required. Cereals contain a wide spectrum of powerful antioxidants, such as phenolic acids, tocotrienols, sterols, and avonoids, inexpensive and nutritionally complementary to fruits and vegetables (Ryan D. et al., 2011). In this respect, whole grains can also be regarded as a good source of naturally occurring phytochemicals (Chen et al., 2004). The main health-promoting components, such as minerals, fibers, vitamins and phytochemicals, including phenolic compounds, are dispensed in wholegrain cereals, but are concentrated in large amounts in the bran (Verardo et al., 2011). Interestingly, oats are commonly consumed as a wholegrain cereal, contrarily to such small grains as rice and wheat. For this, in our traditional processes, we use to grind the whole grains and the bran is not removed. However, in recent years, significant changes have occurred to the milling technology, such as sieving, pearling, and air classification, to obtain refined cereal flour, so that the quality and quantity of nutrients is affected (Ragaee et al., 2013). Among all monocot cereal grains, oats contain a typical class of polyphenols, called avenanthramides (Peterson et al., 2001). These novel alkaloids, the substituted hydroxycinnamic acid conjugates, appear to exert antimicrobial effects and exhibit excellent antioxidant activities, both *in vitro* and *in vivo*, that exceed several times the antioxidant activity of various phenolics, such as vanillin and caffeic acid (Peterson et al., 2001; Sur et al., 2008; Liu S. et al., 2011).

Increased interest in food compounds that have health-enhancing properties requires more information on the phenolic compounds and their contribution to antioxidant capacity in order to encourage the production of new value-added cultivars and lines rich in nutraceuticals and health-beneficial components. For this reason, the present study aims to:

– identify the best method for the extraction of antioxidants and total phenolics from oat grains;

– quantify the total content of compounds with antioxidant properties (total phenols, flavonoids, and carotenoids);

– evaluate the antioxidant activity as radical scavenging and power-reducing activity.

Material and methods

Plant material

The plant material used in this study included two accessions of *Avena magna* Murphy et Terrell., two accessions of *A. murphyi* Ladiz., and six Moroccan hexaploid cultivars (five of *A. sativa* L., and one of *A. nuda* L.; all of them registered with the official national catalogue by the National Institute for Agricultural Research, INRA, Morocco). Besides, sixteen improved hexaploid oat lines, derivatives from interspecific crosses previously achieved by INRA-Morocco, between Moroccan hexaploid oat cultivars of *A. sativa* and wild accessions of the tetraploid oat species *A. magna* and *A. murphyi*, respectively (Table 1). All plant material was harvested during the 2012/2013 cropping season whose rainfall was 571 mm.

Reagents and standards

2,2'-Diphenyl-1-picrylhydrazyl hydrate (DPPH), gallic acid, Trolox, ascorbic acid, the Folin–Ciocâlteu reagent, *β*-carotene, catechin, and extraction solvents used in the study were purchased from Sigma-Aldrich®.

Preparation of oat extracts

We initiated a preliminary test to pinpoint the optimal method for extracting phenolic compounds and antioxidants. Utilizing conventional solvents, like ethanol, methanol and water, widely employed in literature and our lab for polyphenol extraction, we explored four methods to identify the most fitting approach for our unique raw material.

Method A: Duplicates from each ground sample were extracted with 80% ethanol (3 × 33.3 mL, 20 min, with shaking). The first extraction was at 50°C and the further extractions at room temperature. The obtained residue was centrifuged (5 min at 1250×g), after the supernatants were combined and concentrated using the rotary evaporator at 40°C. Phenolic compounds were resolubilized in methanol, filtered through 0.2 µm membranes, and stored at –20°C until the analysis (Peterson et al., 2001).

Method B: Duplicates from each ground sample were extracted with methanol (2 × 50 mL); the sample was put into a test tube over which 40 mL of methanol was poured and vortexed. The tubes were capped and placed into an ultrasound bath at 60°C for 20 min with shaking. After being centrifuged at 7000 rpm for 10 min, the separated liquid was removed and the extraction was repeated once more. The supernatants were combined and concentrated (Dar, Sharma, 2011).

Method C: Duplicates from each ground sample were extracted with methanol (80 mL). The sample were taken and put into conical flasks over which 80 mL of methanol was added and stirred. Then, the microwave extraction system was programmed at 2450 MHZ for 3.5 min. Centrifugation (7000 rpm, 10 min) followed after a 10 min cooldown period. The separated liquid was removed and the residues were mixed with 20 mL of the same solvent, vortexed and centrifuged (7000 rpm, 10 min) again. The supernatants were combined and dried (Dar, Sharma, 2011).

Method D: Duplicates from each ground sample were extracted with water (100 mL). Samples were taken and put into conical flasks over which 100 mL of solvent was added and stirred for 30 min. The sample was centrifuged at 3200×g for 5 min. The resulting supernatant was collected, frozen, and used for the antioxidant analysis (Martinez-Tomé et al., 2004).

Table 1. **List of the studied hexaploid oat cultivars, derivative hexaploid lines from interspecific crosses, and tetraploid accessions of *Avena magna* Murphy et Terrell. and *A. murphyi* Ladiz.**

Таблица 1. **Список изученных гексаплоидных сортов овса, производных гексаплоидных линий от межвидовых скрещиваний и тетраплоидных образцов *Avena magna* Murphy et Terrell. и *A. murphyi* Ladiz.**

Tetraploid accessions		Lines		Hexaploid oat cultivars	
Accessions of *A. murphyi*	Accessions of *A. magna*	Hybrid lines, derivatives of (*A sativa* × *A. magna*) × *A. sativa*	Hybrid lines, derivatives of (*A. sativa* × *A. murphyi*) × *A. sativa*	Cultivar of *A. nuda*	Cultivars of *A. sativa*
P35-42	P1-1	F11-1	F10-1	Bounejemat	Tissir
P50-52	P1-6	F11-2	F10-2		Zahri
		F11-3	F10-3		Soualem
		F11-4	F10-4		Amlal
		F11-5	F10-5		Ghali
		F11-7	F10-6		
		F11-8	F10-7		
			F10-8		
			F10-9		

DPPH free radical scavenging activity

The radical scavenging activity of the samples was measured according to the DPPH assay method (Ndolo, Beta, 2013), modified in our laboratory to measure kinetic parameters. After the blank was adjusted with methanol, volumes of the extracts (0.1 mL) were mixed with 3 mL of 60 µM DDPH methanolic solution. The reaction was monitored by reading absorbance at 517 nm for 60 min at 10 min intervals and until the reaction reached the plateau. A blank reagent was used to study DPPH stability over the test time. The absorbance measured at 30 min was used for the calculation of DPPH scavenged by grain extracts. The antioxidant capacity was measured in methanolic solutions of Trolox in order to express the results. The percentage of absorbance inhibition of the DPPH solution was calculated using the following equation:

$$\text{Radical Absorbance DPPH}(\%) = [(\text{Abst}_{0\ \text{min}} - \text{Abst}_{30\ \text{min}}) / \text{Abst}_{0\ \text{min}}\ \text{min}] \times 100,$$

where $\text{Abst}_{0\ \text{min}}$ is the DPPH absorbance at the time zero, and $\text{Abst}_{30\ \text{min}}$ is the DPPH absorbance after 30 min of incubation.

Ferric reducing antioxidant power assay (FRAP)

The reducing power of grain extracts was determined following the method of M. Oyaizu (1986) and S. Žilić et al. (2011) with a slight modification. The assay medium contained 250 µL of the sample, 200 mM of sodium phosphate buffer (250 µL, pH 6.6), and 1% potassium ferricyanide (250 µL). After incubation in a water bath at 50°C for 20 min, 250 µL of 10% trichloroacetic acid (w/v) was added to the mixture and centrifuged at 10 000 rpm for 10 min. The supernatant (1 mL) was mixed with 1 mL of distilled water and 0.1 mL of ferric chloride solution (0.1%, w/v). The absorbance of the resultant solution was read at 700 nm, and a standard curve was prepared using various concentrations of ascorbic acid, and the reducing power was expressed as mM ascorbic acid equivalents/100 g of material.

Quantification of the total phenolic content (TPC)

Total phenolics were measured according to the Folin–Ciocâlteu procedure (Singleton et al., 1999). One mL of the sample, diluted to 10% of its original concentration with methanol, was mixed with 0.5 mL of the Folin–Ciocâlteu reagent and swirled. After 3 min, 3 mL of sodium carbonate solution (200 mg/mL) was added, mixed, and the reaction was allowed to proceed for 15 min at room temperature. More distilled water was added to the reaction mixtures (10 mL). The sample was then centrifuged for 5 min at 1250×g to remove the white precipitate formed; the absorbance at 725 nm was recorded. A blank sample prepared with 1 mL of methanol in place of the sample solution was used for background subtraction. The total extracted phenolics were expressed as milligrams of gallic acid equivalents GAE/100 g of oat material using a standard curve prepared with gallic acid (25–300 µg/mL).

Quantification of the total flavonoid content (TFC)

The total flavonoid content was assessed according to the colorimetric method described previously by S. Žilić et al. (2011). Appropriate dilutions of sample extracts were reacted with sodium nitrite (0.075 mL, 5%), followed by a flavonoid-aluminum complex formation using aluminum chloride (0.15 mL, 10%). Then, NaOH (0.5 mL, 1 M) was added, and the volume was made up to 2.5 mL with distilled water. The solution was mixed well, and the absorbance was measured at 510 nm against a blank and compared to that of the catechin standards. Flavonoid content was expressed as milligrams of catechin equivalents (CE/100 g of grain).

Quantification of the total carotenoid content (TCC)

Carotenoids were extracted according to the method proposed by E. S. M. Abdel-Aal et al. (2007), and V. U. Ndolo and T. Beta (2013) with some modifications. Briefly, 200 mg of ground samples were mixed with 2 mL of water-saturated butanol in tubes covered with black caps and aluminum foil in a fume hood. The mixtures were vortexed for 30 s and carotenoids extracted by shaking for 15 min at a speed of 40 using a horizontal rotary shaker. After shaking, the samples were left to stand for 60 min at room temperature in the dark and homogenized again before shaking for another 15 min. Lastly, the samples were allowed to stand for another 60 min. About 1.8 mL of the extract were transferred into 2 mL brown micro-centrifuge tubes and

centrifuged at 4000×g and 20°C for 5 min. The supernatants were transferred from microcentrifuge tubes into a quartz cuvette and the absorbance was measured at 450 nm using a UV/Visible spectrophotometer. All the experimental procedures were carried out under dim light, and the extraction tubes were wrapped with black paper to avoid sample degradation by photooxidation. The TCC was expressed as µg *β*-carotene equivalents/g of the sample.

Statistical analysis

Results are expressed as mean values ± standard deviation of three separate measurements in each extract and are reported on a dry matter basis. The significant differences between means were calculated by one-way analysis of variance (ANOVA) using Tukey's studentized range test at $P < 0.05$. Correlations between parameters were examined by Pearson's correlation test.

Results and discussion

Extract yields and extraction methods

Antioxidant capacity of oat lines and cultivars was evaluated on the basis of measuring scavenging activity for the stable 2,2-diphenyl-1-picrylhydrazyl radical by wholegrain extracts (Fig. 1).

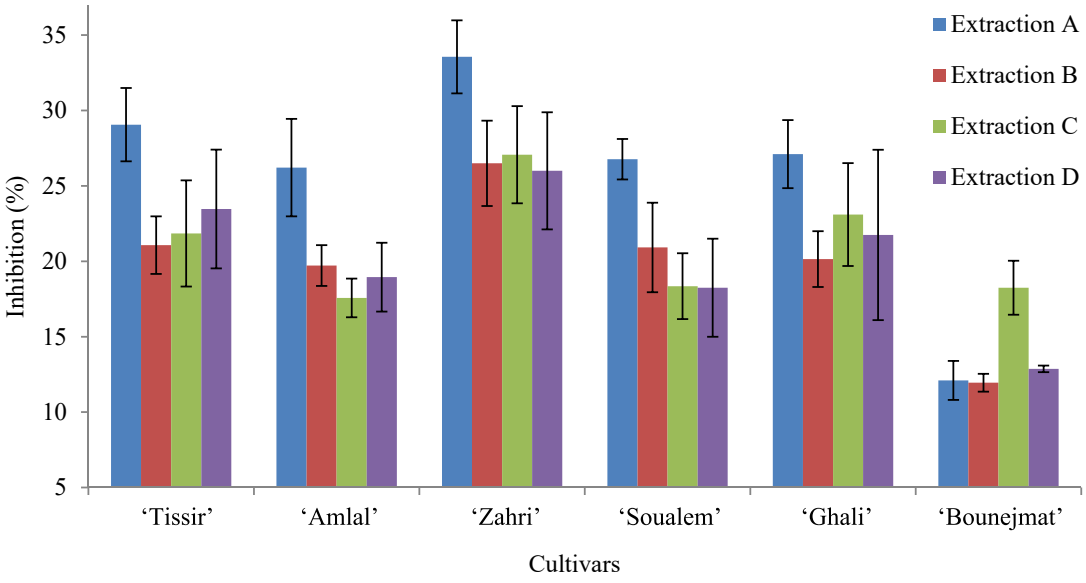

Fig. 1. Scavenging activity of Moroccan oat cultivars extracted according to four different methods, measured by the DPPH assay technique. The data are presented as mean ± SD, n = 3

Рис. 1. Антиоксидантная активность, измеренная с помощью DPPH, у Марокканских сортов овса в экстрактах, полученных четырьмя различными методами. Данные представлены как среднее значение ± стандартное отклонение, n = 3

Under the given experimental conditions, the ethanolic extracts quenched 25.8% of the DPPH radicals on average, whereas the methanolic and aqueous extracts quenched less than 21%. Among the evaluated extraction methods (Table 2), ethanolic extraction (Method A) stands out as the most suitable, consistently delivering high levels of polyphenolics across multiple sources, particularly with 'Zahri', where it achieves a remarkable 51.52% ± 2.59. Ethanolic extraction showcases its effectiveness in various sources, emphasizing its versatility. However, it is crucial to note that optimal performance may vary depending on the specific source. For instance, methanolic extraction (Method B) performs well with 'Tissir', yielding 37.67% ± 1.08, but demonstrates mixed results with other sources. Similarly, methanolic extraction (Method C) generally falls in the midrange, with an example of 'Zahri' at 38.6% ± 0.76. Interestingly, aqueous extraction (Method D) consistently provided lower polyphenolics and yield percentages, such as 27.32% ± 1.66 for 'Tissir'. These findings align with previous research that has examined different methods of extracting polyphenols from oat (Chmelová et al. 2015; Escobedo-Flores et al. 2018; Sridhar et al. 2021).

Thus, the extracts obtained by this method were selected for further studies and ethanol was used as an extraction solvent for the rest of samples.

Total phenolic content

Total phenolics varied widely in the material investigated in this study and ranged from 23.1 to 56.5 expressed as milligrams of gallic acid per one hundred grams of dry matter (Fig. 2). The highest amounts of phenolics extracted by Method A were originated from both *A. magna* and *A. murphyi* species; lines $F_{11\text{-}5}$ (magxsat) and $F_{10\text{-}3}$ (murxsat) were noticeable with (54.83 ± 1.6) mg/100 g and (53.26 ± 2.05) mg/100, respectively. Past research conducted in a similar attempt fits in this profile (Brindzova et al., 2008; Alrahmany, Tsopmo, 2012). However, when each species had been examined, *murphyi*'s extracts showed a statistically significant difference in terms of TPC compared to *magna*'s extracts.

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Total flavonoid content

The flavonoid content of oat lines and cultivars was expressed as milligrams of catechin equivalents per 100 grams of grain (Fig. 3). The TFC of the samples was relatively significant and ranged from 14.4 ± 0.87 to 30.2 ± 2.02 mg CE/100 g. Cv. 'Zahri', accessions *A. magna* $P_{1\text{-}1}$ and *A. murphyi* $P_{35\text{-}42}$, as well as line $F_{10\text{-}3}$ (murxsat) contained significant amounts of flavonoids compared to the analyzed cultivars, the obtained values being in line with the findings by H. Y. Kim et al. (2013), but about twice higher than those by S. Žilić et al. (2011). In addition, R. A. Carciochi et al. (2014) stated that high yields were obtained with ethanol and reached a maximum in the region with close to 80% ethanol.

Table 2. **Polyphenolic content and extraction yields of the extracts obtained from Moroccan oat cultivars**

Таблица 2. **Содержание полифенолов и полнота извлечения целевых веществ, полученных из марокканских сортов овса**

Cultivar	Method	Polyphenolics[a]	Yield (%)
'Tissir'	A	38.17 ± 0.85	6.75 ± 0.42
'Amlal'	A	40.65 ± 1.17	5.39 ± 0.54
'Zahri'	A	51.52 ± 2.59	6.56 ± 0.36
'Soualem'	A	39.50 ± 1.42	4.08 ± 0.24
'Ghali'	A	39.37 ± 0.96	7.88 ± 0.33
'Bounejmat'	A	33.75 ± 2.18	3.07 ± 0.7
'Tissir'	B	37.67 ± 1.08	4.99 ± 0.19
'Amlal'	B	32.85 ± 0.71	3.76 ± 0.33
'Zahri'	B	39.50 ± 1.00	5.58 ± 0.74
'Soualem'	B	32.47 ± 1.68	3.81 ± 0.80
'Ghali'	B	29.32 ± 1.90	6.68 ± 0.94
'Bounejmat'	B	27.07 ± 0.71	3.29 ± 0.81
'Tissir'	C	33.15 ± 2.67	4.73 ± 0.62
'Amlal'	C	27.97 ± 1.96	3.33 ± 0.50
'Zahri'	C	38.60 ± 0.76	3.63 ± 0.64
'Soualem'	C	28.50 ± 0.93	3.17 ± 0.16
'Ghali'	C	29.50 ± 0.90	5.53 ± 0.60
'Bounejmat'	C	25.97 ± 1.89	2.10 ± 0.28
'Tissir'	D	27.32 ± 1.66	4.01 ± 0.20
'Amlal'	D	27.70 ± 2.12	3.90 ± 0.41
'Zahri'	D	29.45 ± 3.12	5.70 ± 0.64
'Soualem'	D	23.80 ± 1.91	3.53 ± 0.62
'Ghali'	D	27.47 ± 1.70	4.86 ± 0.28
'Bounejmat'	D	25.42 ± 1.68	2.16 ± 0.51

Note: [a] – mean of triplicate measurement ± SD expressed as mg gallic acid equivalents per 100 g of grain (weight basis); A – extraction with ethanol solvent; B – extraction with methanol solvent; C – extraction with methanol solvent in a microwave system; D – extraction with water solvent

Примечание: [a] – среднее значение трехкратного измерения ± SD, выраженное в мг эквивалента галловой кислоты на 100 г зерна (массы); A – экстракция этанолом; B – экстракция метанолом; C – экстракция метанолом в микроволновой системе; D – экстракция водой

Total carotenoid content

Water-saturated 1-butanol was chosen for this experiment due to its common use in determining the total yellow pigment content for selecting intense amber in grain flours as well as in evaluating the quality and quantity of carotenoids. The total carotenoid content in grains, expressed as μg *β*-carotene equivalents per one gram of the sample, was relatively low in all tested samples (Fig. 4). The TCC mean values ranged from 1.39 ± 0.1 μg/g to 4.17 ± 0.2 μg *β*-carotene equivalents/g and showed genetic diversity among oat lines in their content of carotenoids. Additionally, significant variability within oat species was observed, as indicated by the high coefficient of variability values (Žilić et al., 2011).

Carotenoid extracts were also examined for their antioxidant activity using the DPPH assay method, and they exhibited significant antioxidant capacity, which indicated their contribution to the total antioxidant activity. A high and positive correlation between %DPPH scavenging and total carotenoids was found, pointing to their contribution to antioxidant activity (Fig. 5). Similar facts were observed by S. Žilić et al. (2011). There was a discernible variation across the samples, with accessions P35-42 and P50-52 showing lower antioxidant activities, starting at approximately 15%. Hybrid lines displayed a wider range of activity, with F11-3 peaking at nearly 45%, suggesting a significant presence of antioxidant carotenoids. Among the cultivars, 'Amlal' and 'Zahri' also

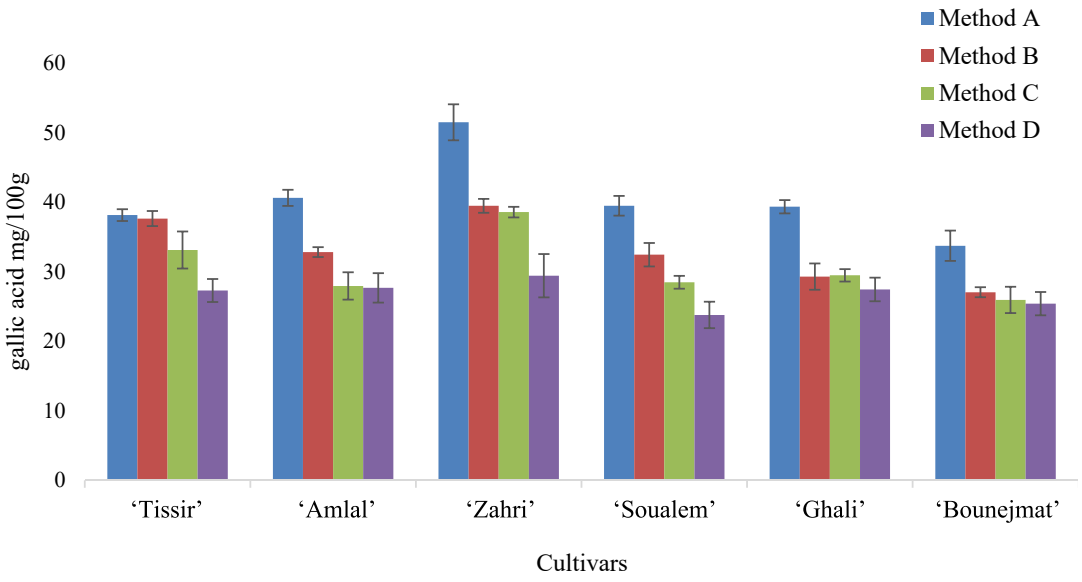

Fig. 2. **Concentration of total phenolics in Moroccan oat cultivars, extracted by four different methods, expressed as gallic acid equivalents (mg GA/100 g). Data are presented as mean ± SD, n = 3**

Рис. 2. **Концентрация общих фенольных соединений в марокканских сортах овса в экстрактах, полученных четырьмя различными методами, выраженная в эквиваленте галловой кислоты (мг ГК/100 г). Данные представлены как среднее значение ± стандартное отклонение, n = 3**

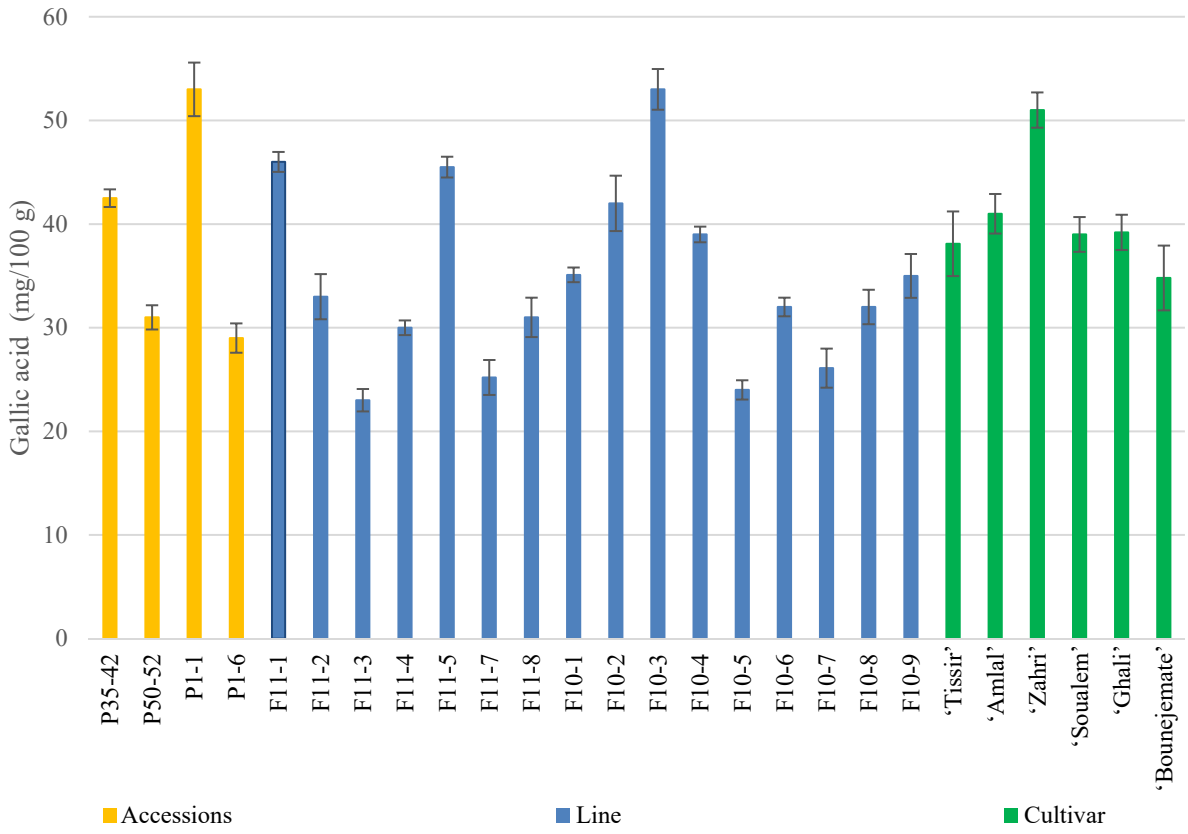

Fig. 3. **Concentration of total phenolics in the grain of oat lines and cultivars expressed as gallic acid equivalents (mg GA/100 g). Vertical bars represent the standard deviation of each data point. Values are mean ± SD (n = 3)**

Рис. 3. **Общее содержание фенольных соединений в зерне линий и сортов овса, выраженное в эквиваленте галловой кислоты (мг ГК/100 г). Вертикальные столбцы соответствуют средним значениям ± стандартное отклонение (n = 3)**

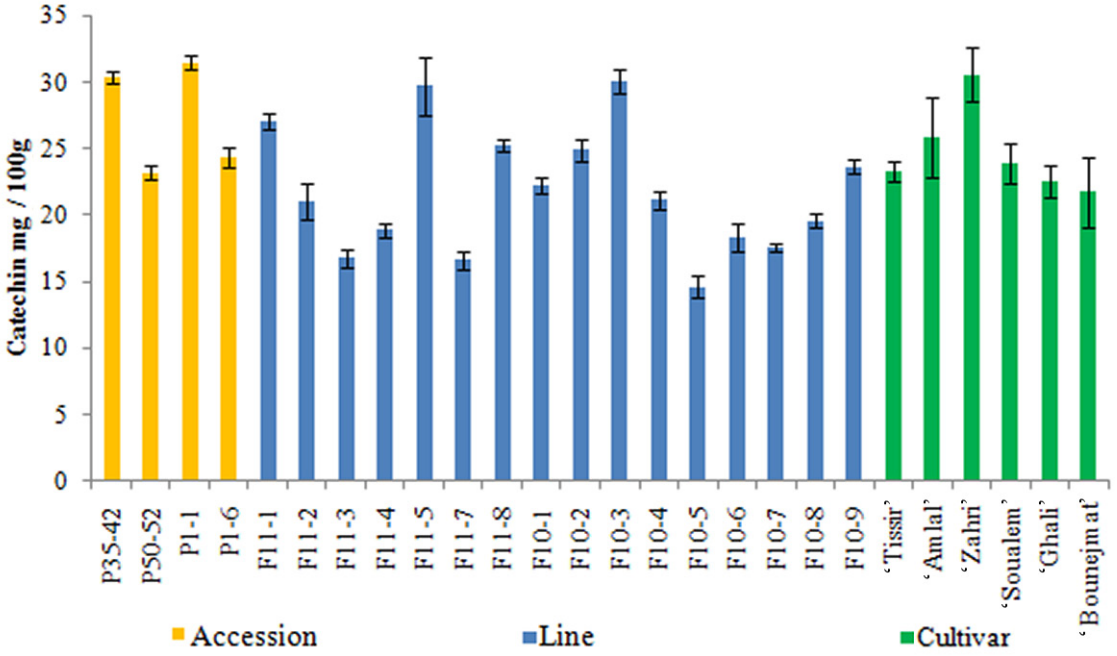

Fig. 4. Concentration of flavonoids in the grain of oat lines and cultivars expressed as catechin equivalents (mg C/100 g). Vertical bars represent the standard deviation of each data point. Values are mean ± SD (n = 3)

Рис. 4. Концентрация флавоноидов в зерне линий и сортов овса, выраженная в катехиновом эквиваленте (мг С/100 г). Вертикальные столбцы соответствуют средним значениям ± стандартное отклонение (n = 3)

reached higher values, close to 40%, indicating a rich carotenoid profile.

These variations reflect inherent genetic diversity and align with findings from other studies indicating that certain oat genotypes possess enhanced antioxidant properties due to their carotenoid composition (Emmons, Peterson, 2001). Such data are crucial for breeding programs focused on improving the health benefits of oats, as supported by published sources where dietary antioxidants are correlated with a reduced risk of chronic diseases (Willcox et al., 2004).

Antioxidant activity

Antioxidant properties of ethanolic extracts were evaluated by measuring scavenging activity for DPPH radicals; the total antioxidant activities in grain were expressed as millimoles of Trolox equivalents per one hundred grams of grain. The DPPH assay measures the reducing ability of antioxidants towards the DPPH radical through discoloration, showing the percentage of DPPH that has been quenched. There was a wide and significant variation in %DPPH scavenging activity among the cultivars and lines, but all samples had considerable antioxidant activities, ranging from 0.496 to 1.046 mM TE/100 g (dm), on average (see Fig. 5). The greatest potential for antioxidant activities was observed in lines $F_{11\text{-}5}$ (magxsat) (1.046 ± 0.04) mM TE/100 g, $F_{10\text{-}3}$ (murxsat) (0.96 ± 0.15) mM TE/100 g, and the accessions of *A. magna* $P_{1\text{-}1}$ (0.97 ± 0.04) mM TE/100 g. These results were consistent with those reported by C. Serea and O. Barna (2011).

Reducing power

The reducing power of oat extracts is presented in Figure 6. The reducing power in different lines significantly ($P < 0.0001$) differed from one another. The highest reduction power was detected in the tetraploid accessions of *A. magna* and *A. murphyi*, and cvs. 'Ghali' and 'Bounejmat', with the following descending order: *A. magna* $P_{1\text{-}1}$ > 'Ghali' > *A. murphyi* $P_{50\text{-}52}$ > 'Bounejmat'. In this method, the ferric/ferricyanide complex is reduced to ferrous form depending on the presence of antioxidants (Choi, et al., 2007). Besides that, P. D. Duh (1998) stated that the reducing power of a compound is attributed to its hydrogen-donating ability (Duh, 1998). Our results on the reducing power demonstrate the electron donor properties of oat extracts thereby terminating free radical reactions.

The graph (Fig. 7) presents the ferric reducing antioxidant power (FRAP) of various oat lines and cultivars, expressed as ascorbic acid equivalents (mM AA/100 g). P1-1 leads with the highest FRAP value, nearing 3 mM AA/100 g, indicative of a potent antioxidant capacity. The hybrid lines and cultivars generally showed moderate FRAP values, with a mean around 1.5 mM AA/100 g, with the exception of 'Ghali', which displayed an elevated antioxidant capacity close to 2.5 mM AA/100 g.

Relationships between phytochemical contents and antioxidant activity determined by DPPH radical scavenging and reduction power methods are presented in Table 3. The content of total phenolics and DPPH scavenging activity are in agreement (r = 0.798), indicating a strong link between phenolic compounds and antioxidative capability (see Table 3).

C. Serea and O. Barna (2011) also noted a highly positive correlation between TPCs and antioxidant activity of the phenolic extracts but C. L. Emmons et al. (1999) reported no significant correlations for this item, as different responses of phenolic compounds in the Folin–Ciocâlteu method could be provided (Emmons et al., 1999; Serea, Barna, 2011). Likewise, flavonoids are an important class of phenolic compounds found in oat with various potential mechanisms of action and effects on human health. They are considered a good radical scavenger due to the presence of polyhydroxyl groups in their structure which may explain the relationship observed between total flavonoids, total phenolics (r = 0.848), and antioxidant activity (r = 0.749). Regarding the relationship between TCC and DPPH radical scavenging activity, highly significant correlations were found (r = 0.891). This may be attributed to the properties of carotenoids which act as radical scavengers and singlet oxygen quenchers (Leen-

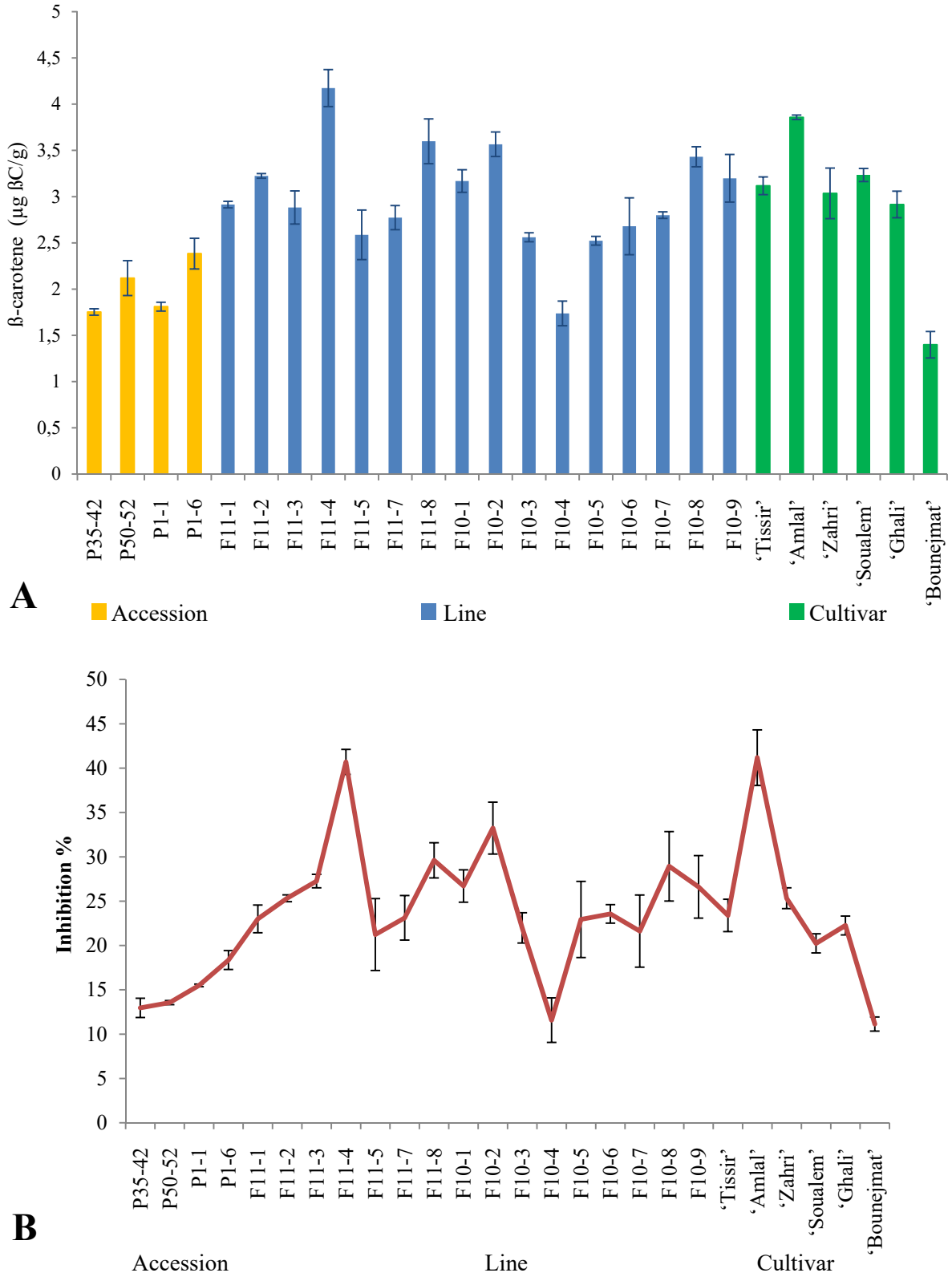

Fig. 5. Concentration of carotenoids in the grain of oat lines and cultivars expressed as *β*-carotene equivalents (µg *ß*C/g) (A), and its induced inhibition (B). Vertical bars represent the standard deviation of each data point. Values are mean ± SD (n = 3)

Рис. 5. Концентрация каротиноидов в зерне линий и сортов овса, выраженная в эквиваленте β-каротина (мкг ßC/г). "A" и его индуцированное торможение "B". Вертикальные столбцы соответствуют средним значениям ± стандартное отклонение (n = 3)

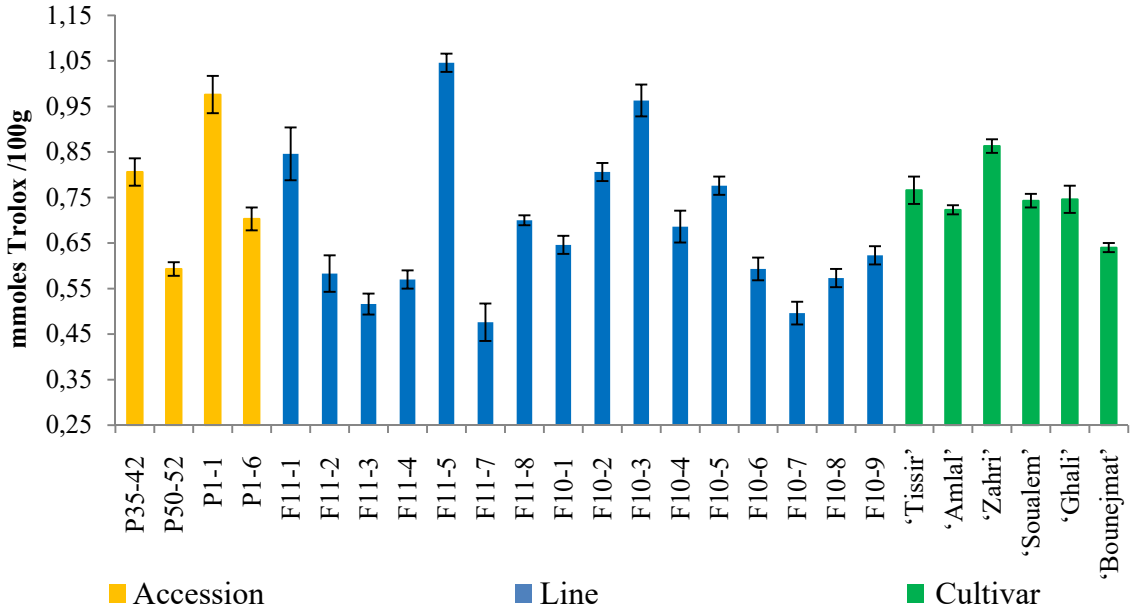

Fig. 6. Free radical scavenging activity of oat lines and cultivars analyzed by DPPH method, expressed as Trolox equivalents (mM T/100 g). Vertical bars represent the standard deviation of each data point. Values are mean ± SD (n = 3)

Рис. 6. Антиоксидантная активность линий и сортов овса, проанализированная методом DPPH, выраженная в эквиваленте тролокса (мМ Т/100 г). Вертикальные столбцы соответствуют средним значениям ± стандартное отклонение (n = 3)

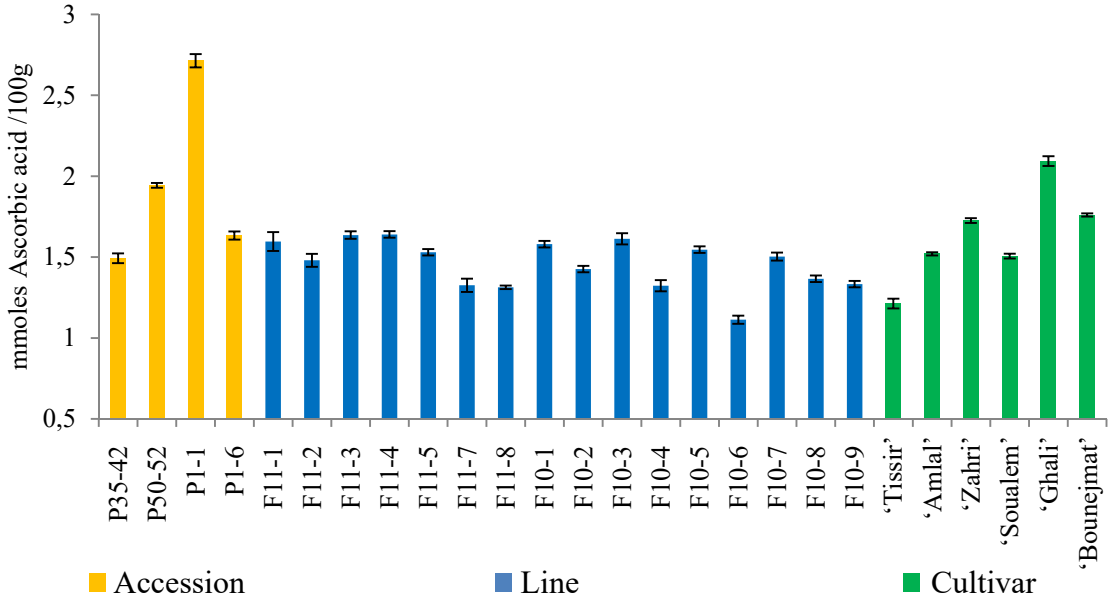

Fig. 7. Ferric reducing antioxidant power of oat lines and cultivars, expressed as ascorbic acid equivalents (mM AA/100 g). Vertical bars represent the standard deviation of each data point. Values are mean ± SD (n = 3)

Рис. 7. Антиоксидантная активность линий и сортов овса, проанализированная методом FRAP, выраженная в эквиваленте аскорбиновой кислоты (мМ АК/100 г). Вертикальные столбцы соответствуют средним значениям ± стандартное отклонение (n = 3)

hardt et al., 2006). In fact, V. U. Ndolo and T. Beta (2013) suggested that this correlation could be explained by lutein and zeaxanthin contents extracted using the water-saturated butanol (Ndolo, Beta, 2013). In contrast, Y. Choi et al. (2007) stated that no relationship was found between antioxidant activity and carotenoid content (Choi et al., 2007).

In light of the roles that carotenoids play in promoting the health of eyes and skin (Abdel et al., 2007), such trait is regarded as one of the main criteria for assessing the commercial and nutritional value of flours, possessing additional features that may hold potential as natural functional food ingredients.

The FRAP values were shown to correlate slightly (0.331) with the DPPH radical scavenging activities, but no noticeable relation was found between FRAP and the studied traits.

To determine which ratios and solvents are to be used in this experiment, previous research was consulted and complemented with the data generated from this experiment

Table 3. Correlations between total phenolics, flavonoids and carotenoids, and DPPH scavenging activity, as well as reducing power and DPPH scavenging activity in oat lines and cultivars

Таблица 3. Коэффициенты корреляция между общим содержанием фенолов, флавоноидов и каротиноидов и антиоксидантной активностью, измеренной с помощью метода DPPH и FRAP у линий и сортов овса

	FT	PT	CT	IC	DPPH	FRAP
FT	1.00	0.848**	–0.120	–0.137	0.749**	0.360*
PT		1.00	–0.105	–0.102	0.798**	0.194
CT			1.00	0.891**	–0.197	–0.334
IC				1.00	–0.174	–0.257
DPPH					1.00	0.331*
FRAP						1.00

Note: ** – significant at $P < 0.001$,* – significant at $P < 0.01$, and all others at $P > 0.05$; FT – total flavonoid content; PT – total phenolic content; CT – condensed tannin content; IC – inhibitory concentration; DPPH – antioxidant activity measured by the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay; FRAP – antioxidant power measured by the ferric reducing antioxidant power assay

Примечание: ** – значимо при P < 0,001,* – значимо при P < 0,01, все остальные – при P > 0,05; FT – общее содержание флавоноидов; PT – общее содержание фенолов; CT – содержание конденсированных танинов; IC – ингибирующая концентрация; DPPH – антиоксидантная активность, измеренная с помощью анализа DPPH (2,2-дифенил-1-пикрилгидразила); FRAP – антиоксидантная способность, измеренная с помощью анализа железо-восстанавливающей антиоксидантной способности

(Przybylski et al., 1998; Zieliński, Kozłowska, 2000; Carciochi et al., 2014). The outcomes of this study demonstrate that ethanol is an effective solvent in extracting phenolics, flavonoids, and other polar substances in oat. With this, 80% ethanol extracts were used for measuring total phenolics, flavonoids, and antioxidant activity. All the same, it was not possible to compare our lines and cultivars directly with the published data, but an estimate of their antioxidant capacities was provided for screening and comparing them with other oat genotypes originated from different ecogeographic areas. In general, most reports indicated that phytochemical contents in whole grains were found to be high, and great shares of their beneficial characteristics were attributed to their antioxidant compounds (Slavin, 2003; Ryan L. et al., 2007). It must be pointed out that the used methods at best extracted only the free or loosely attached and more readily soluble phenolic compounds in the tested material and did not extract phenolic compounds tightly bound to cell wall materials (Liu R.H., 2007), so that the real contents are underestimated. Additional research is needed to investigate bound phenolics, since 75% of oat's phenolics are in the bound form (Liu, 2007), and evaluate their contribution to the total antioxidant activity as well as their composition.

As a secondary objective, this experiment was conducted to highlight that Moroccan oat germplasm resources, and particularly hexaploid oats, attracted much attention for their genetic characteristics (Ladizinsky, 2012). In the main, the presented results revealed that Moroccan oat cultivars and lines demonstrated high content of phenolics, flavonoids and carotenoids, and manifested potent antioxidant activity.

The comparison in the multiple range test revealed a highly significant difference ($P < 0,01$) between the released lines and both tetraploid and hexaploid parents for the studied traits; the extracts from different oat genotypes differed significantly in their radical scavenging capacities against DPPH and in TPC, indicating the potential effect of the genotype on the antioxidant properties in oat. C. L. Emmons and D. M. Peterson advanced this fact and demonstrated that the cultivar and location affected phenolic contents and antioxidant activities (Emmons, Peterson, 2001). In addition to this variation, it is worthy to mention further variation for phenolics and carotenoids observed among the lines released from *A. magna* and *A. murphyi* species.

Based on these results and previous research (Manzali et al., 2016, 2017, 2023), these lines and cultivars rank on the top of functional foods rich in phytochemicals, with overwhelming evidence indicating that the regular consumption of products rich in phytochemicals is efficient in promoting health effects against such maladies as cancer, chronic diseases, etc. (Ragaee et al., 2006; Liu R.H., 2007; Van Hung, 2016).

Conclusion

Starting with the optimal approach for extracting polyphenolics from oats, our results consistently emphasize that ethanolic extraction (Method A) stands out as the most effective method. Superior performance of this method across diverse oat genotypes is evident in its consistent yield of high polyphenolic content levels, particularly noteworthy with cv. 'Zahri' (51.1 mg/100 g). This not only reaffirms ethanolic extraction as the preferred option for obtaining extracts with substantial antioxidant capacity but also underscores its robust ability to capture the complete range of antioxidant compounds present in Moroccan oat germplasm.

Following the recorded values for all oat extracts, we can ascertain the high phenolic content and antioxidant potential in Moroccan oat germplasm due to a wide range of antioxidant compounds. This study provided a comparison of the content of phytochemicals in a set of selected lines and cultivars grown under the same agronomic and environmental conditions. Substantial variation was demonstrated in the content values, indicating that there is sufficient genetic variation in the content of antioxidant components to be exploited by breeders to develop cultivars with enhanced quality for human nutrition. Additional research is needed to further investigate phenolic compounds, individual carotenoids, and phytoestrogen, and evaluate their contribution to the total antioxidant activity of oat and its nutraceutical value. Even when present in small amounts, they should be taken into ac-

count in order to understand/validate their bioactivity and bioavailability. In spite of this fact, these results provide an improved understanding of oat potential and identify the high-quality extracts of these lines that can be acquired for the use in oat breeding as alternative sources of natural antioxidants and other bioactive compounds in the formulation of functional foods.

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