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Phytochemical Characterization of Hydroponic Naked Barley, Analysis of Antioxidant Potential

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ABSTRACT

Background: In the present quantitative and qualitative study, to compare the new variety of naked barley with other cereals (durum wheat, commun wheat, coated barley). Regarding the richness in phytonutrients including mainly flavonoids, polyphenols, tannins condensed in a hydroponic culture.

Methods: The experiment was conducted in Laboratory of life sciences and techniques, Souk Ahras University in Algeria during 2019-2022. The antioxidant power was tested according to the DPPH free radical scavenging method. The results obtained from the quantitative study of methanolic extracts show that varieties rich in tannins and flavonoids have a significant antioxidant activity than a variety rich only in total polyphenols. Qualitative studies based on HPLC of these compounds allow us to visualize phenolic traces of our extracts, this analysis showed that our extracts mainly contain flavonoids of the flavone and flavonol type, potentially interesting for their biological properties.

Result: The results reported the existence of a very highly significant difference ($P < 0.001$) between IC_{50} of the new variety Chaiir Ennabi (Naked Barley) compared to ascorbic acid, which shows a lower antioxidant activity of this variety.Barley variety Barbarous (coated barley) had the highest antioxidant activities and the highest contents of phenolic compounds.

Key words: Antioxidant power, Condensed tannins, Flavonoids, HPLC, Naked barley, Polyphenols.

INTRODUCTION

In developing countries, cereals and their derivatives constitute the primary source of food, in particular in Maghreb countries. In Algeria, they still represent the core of the nutritional system and a fundamental guarantor of the national economy.

Naked barley has an interesting richness in functional ingredients. According to epidemiological studies. Diets high in whole barley flour fight hyperlipidemia, diabetes and atherosclerosis (Guo *et al*., 2018). Chaiir Ennabi is among the rarest barley varieties in the world. It has just been rediscovered in the wilaya of Souk-Ahras Algeria in 2010 at the daïra de Merahna. This variety, unknown in Algeria, was considered as a possible nutritional source for both humans and livestock. Hence several regional and national agronomic studies aim to determine its yield, reveal its resistance to abiotic stresses and evaluate its phytochemical characteristics and its biological activities.

Recently the interest of scientific studies is directed more and more towards the aerial part of cereals. These studies show that the leaves of these species are rich in phenolic components. This gives cereals a strong antioxidant function (Abdel *et al*., 2006; Jonnala *et al*., 2010). Barley was considered a good source of phenolic compounds, such as cinnamic and benzoic acid derivatives, proanthocyanidins, flavonols, flavanones, flavones, which can potentiate the antioxidant and antitumor action, lowering blood lipids and hypoglycemia (Shen *et al*., 2016, Zhu and Xu, 2015).

This research is aimed to compare the new variety of naked barley with other cereals. Regarding the richness of phytonutrients in hydroponic culturs in order to reveal new phytogenetic sources that are rich in phenolic compounds. As a part of quality improvement of the leaves of these cereals in Algeria.

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MATERIALS AND METHODS

The experiment was conducted in Laboratory of life sciences and techniques, Institute of Agriculture and Veterinary Sciences, Souk Ahras University in Algeria (36°13′N, 8°11′E and 887 m above sea level) during 2019-2022. We used as vegetal material nine varieties of cereals: three durum wheat (*Triticum durum* Desf.) varieties, three commun wheat [*Triticum aestivum* (L.) varieties], two coated barley varieties and one naked barley (*Hordeum vulgare* L.) variety, the grains were supplied by the (ITGC) of EL-Khroub-Constantine (Table 1).

The seeds of nine (09) samples were sown on December 2020 in rectangular hydroponics tanks. Aeration of the nutrient solution was carried out using aquarium pumps with a flow rate that allowed good aeration without causing agitation of the root system. All the experiments were carried out in a controlled culture chamber where the temperature is 20°C during the day and 15°C at night, the humidity is 75%, under a bright ceiling with an illumination intensity of 500 µmol m^{-2} s^{-1} and a photoperiod of 16 h. The methanolic extracts (methanol/water: 70/30) were prepared by the technique described by Kondakova (2009). The determination of total phenols is carried out by Folin-ciocalteu according to the method of Zhu *et al.* (2011). The flavonoids contained in the methanolic extracts are estimated by the $AlCl_3$ method (Ayoola *et al*, 2008).

The condensed tannins were assayed by colorimetry using the method described (Djeridane *et al*., 2006). The anti-free radical activities of the extracts are measured according to the method described by Kirby and Schmidt (1997). HPLC stands for either high performance liquid chromatography or high pressure liquid chromatography. This technique is used for the separation, identification and measuring organic compounds. The sample is entrained by a solvent passing through a chromatographic column. The presence of a product is detected at the outlet of the column. The analysis is carried out by SHUMADZU brand HPLC equipment at the laboratory of the research and technic center of physicochemical analysis (CRAPC Ouargla Algeria). All statistical analyzes were processed by SPSS statistical software version 26 (IBM SPSS, 2019). Normality is verified by the Shapiro-Wilk test across all selected parameters; contents of total polyphenols, flavonoids, tannins, DPPH test, IC_{50} in order to choose the right parametric or non-parametric test. Test (PCA) is applied in order to highlight the possible relations between the different variables (contents of a few secondary substances such as total polyphenols, flavonoids and tannins) with the antioxidant activity within the varieties and also with the new variety of naked barley. A Hierarchical Ascending Classification (HAC) is done as a complementary analysis to identify the number and composition of each homogeneous group of varieties.

RESULTS AND DISCUSSION

The MANOVA test showed a very highly significant difference ($F = 901.85$; $P<0.001$) concerning the content of total polyphenols within the varieties. The Barbarous variety (Barley br) recorded the highest content (95.92 mgEAG/ml). On the other hand, the varieties Ain lahma (durum wheat al), Akhamokh (Common wheat ak) and Boumerzoug (Common wheat bm) have respectively the lowest levels (17.30; 21.79 and 35.40 mgEAG/ml). Cirta (Durum Wheat c), Chaiir nnabi (naked barley NB), Massine (Common Wheat m), Saida (Barley s) and Wahbi (Durum Wheat w) varieties have been reported with varying intermediate and ascending levels of 42.94 to 88.38 mgEAG/ml (Fig 1).

The phenolic content of the methanolic extracts of the plant is subject to several variations linked directly to the origin of the plant as well as to intrinsic (genetic) and extrinsic factors (climatic condition; maturity at harvest). Not to mention the disposition of the plant to various diseases that can alter its integrity (Zeghad, 2009). According to Khalfallah (2013) and Adoum 2002, the genetic, structural or physiological factors of cereals are the decisive elements and the cause of this variation.

Table 1: The tested varieties and their origins.

Varieties	Wahbi durum wheat	Cirta durum wheat	Ain lahma durum wheat	Massin commun wheat	Akamoukh commun wheat	Boumerzogue commun wheat	Saida barley	Barbarose Barley	Chair ennabi naked barley
Origins	Algeria	Algeria	Algeria	CIMMYT	CIMMYT	CIMMYT	Algeria	France (INRA)	Saoudi Arabia

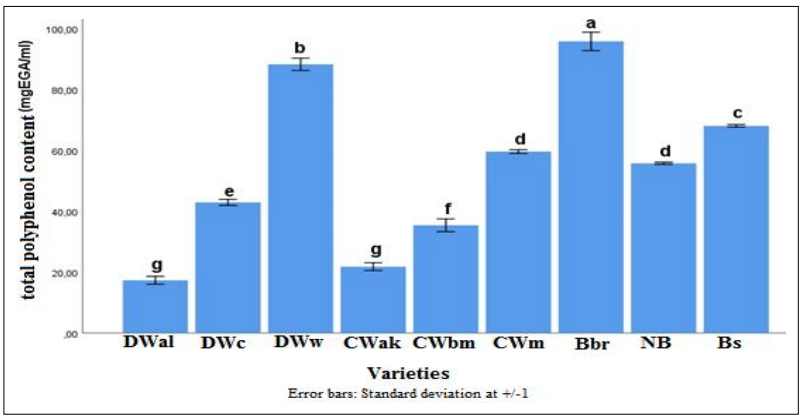


Fig 1: Total polyphenol contents in nine cereal varieties.

Flavonoids are among the highly reactive phenolic compounds as hydrogen and electrons. They are therefore excellent antioxidant compounds (Cotelle, 2001). The MANOVA statistical test reported a very highly significant difference (F = 470.36; P<0.001) in flavonoid contents between the varieties tested. The Barbarous variety (Barley br) presented the highest content (59.04 mgEQU/ml). On the other hand, the lowest levels were recorded in the Akhamokh (Common wheat ak), Wahbi (Durum Wheat w) and Ain Lahma (Durum Wheat al) varieties (with averages varying from 11.21 to 13.69 mgEQU/ml). The Boumerzoug (Common wheat bm), Cirta (Durum Wheat c), Chaiir Ennabi (naked barley NB), Massine (Common wheat m) and Saida (Barley s) varieties were given intermediate levels varying from 18.52 to 35.48 mgEQU/ml (Fig 2).

The phenolic content and particularly the flavonoids present very diverse results, this diversity can be explained by the association of the total phenolic content and in particular that of the flavonoids with the genotype of the seed (Vaher *et al*., 2010; Verma *et al*., 2009; Stalikas, 2007). At the same time, the influence of growth conditions on the biosynthesis and accumulation of phenolic compounds has been proven by Vaher *et al.* (2010).

The MANOVA test reported a very highly significant difference (F = 449.84; P<0.001) between the tannin contents of the tested varities. The highest content was found in the Massine variety (Common Wheat m) (11.01 mgEGA/ml) and even the Barbarous variety (Barley br) has a high content, it is around 8.32 mgEGA /ml. The Ain lahma (durum wheat al) and Boumerzoug (commun wheat bm) varieties showed the lowest levels; the averages are 1.94 and 2.95 mgEGA/ml respectively. The tannin levels in the varieties Chaiir Ennabi (Naked Barley), Akhamokh (Common wheat ak), Wahbi (Durum wheat BDw), Saida (Barley Os) and Cirta (Durum wheat BDc) are intermediate, varying from 3.65 to 6 .01 mgEGA/ml (Fig 3).

At all the tested concentrations, the methanolic extracts of the varieties significantly inhibited the DPPHÿ radical in a dose-dependent manner (P<0.01 vis-à-vis the negative control), the Pearson correlation coefficient varied from 0.97 to 0.99, which indicates the existence of the strong positive correlations of all the relations (Table 2) (Fig 5).

The extract of the Cirta variety (Durum wheat c) showed the highest activity among all the extracts tested with an inhibition percentage of 76.71% reached at the concentration of 100 µg/ml. Similarly, the extract of the Barbarous variety (Barley br) with a high percentage of inhibition (76.49%). The extracts of the varieties Boumerzoug (Common wheat bm), Akhamokh (Common wheat ak) and Ain lahma (Durum wheat al) recorded low percentages of inhibition varied from

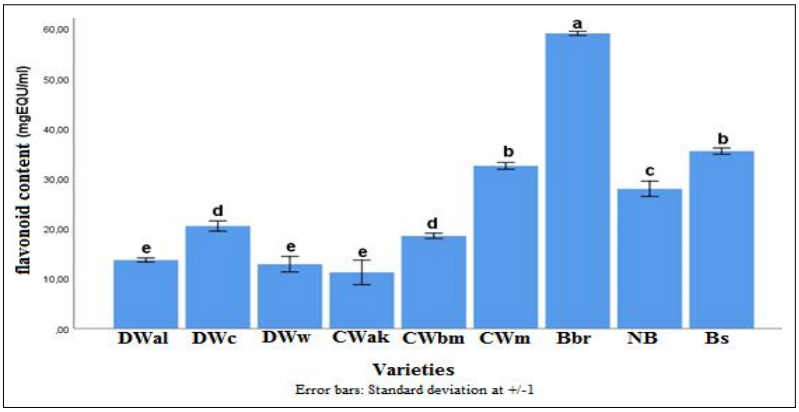


Fig 2: Flavonoid content in nine cereal varieties.

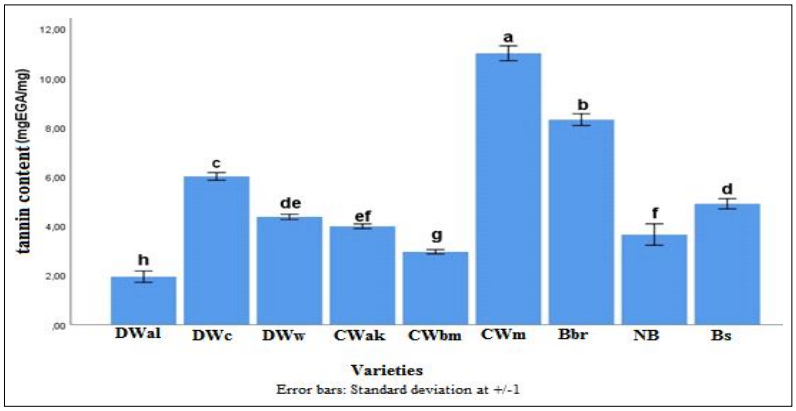


Fig 3: Tannin levels in nine cereal varieties.

50.33% to 55.32%. The rest of the varieties like Saida (Barley s), Wahbi (Durum wheat w), Chaiir ennebi (Naked Barley) and Massine (Common wheat m) have average antioxidant activities with a percentage of inhibition varied from 55.57% to 63.77%. It is noted that across all the tested extracts, the percentages of inhibition evolved gradually with the concentrations used 20; 25; 50; 75; 80 and 100 µg/ml. All extracts reached their maximum activity at 100 µg/ml. The ascorbic acid used as a reference inhibited the DPPH radical by 90.04% at 100 µg/ml (Fig 4; Fig 5).

Study of the antioxidant activity of methanolic extracts from cereal leaves. According to the free radical scavenging method DPPH has shown that methanolic extracts possess moderate antioxidant activity. These extracts could therefore constitute an alternative to certain synthetic additives. It is therefore very likely that they contain compounds which, once purified, can exhibit an activity comparable to that of ascorbic acid.According to the circle of correlation established by the ACP, the antioxidant activity (DPPH or the percentage of inhibition) is more correlated with the contents of tannins and flavonoids than the total polyphenols. These results are consistent with those of Đordevic′ and Dimitrijevic′-Brankovic (2010) who showed that there was a weak correlation between TPC and DPPH radical scavenging activity in cereals (Fig 6).

Cereals with higher TPC values were not necessarily better at inhibiting DPPH. According to Brand-Williams *et al*. (1995), ferulic acid, the main phenolic acid in cereal leaves, showed a weak radical-scavenging effect in experiments with the radical DPPH, which may explain the discrepancies.

In our study, the TFC of the extracts corresponds well to the TPC ($r = 0.668$, $p<0.01$). This indicates that flavonoids are the major phenolic compounds present in the cereal leaf extract. However, CCT correlated moderately with TPC ($r = 0.513$, $p<0.01$). This indicates that condensed tannins could be a second phenolic compound present in cereal

Table 2: Linear correlation between the concentrations of the negative control, the ascorbic acid and the methanolic extracts of nine varieties of cereals.

Extract	Vit.C	DWal	DWc	DWw	CWak	CWbm	CWm	Bbr	NB	Bs
Control	0,75	0,97**	0,99**	0,99**	0,98**	0,98**	0,98**	0,98**	0,98**	0,98**

**The correlation is significant at the 0.01 level.

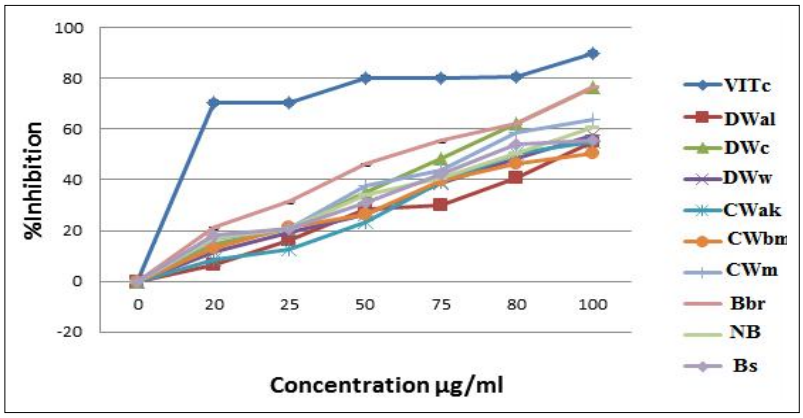


Fig 4: Percentage of DPPH radical inhibition by extracts of nine varieties of cereals as well as ascorbic acid.

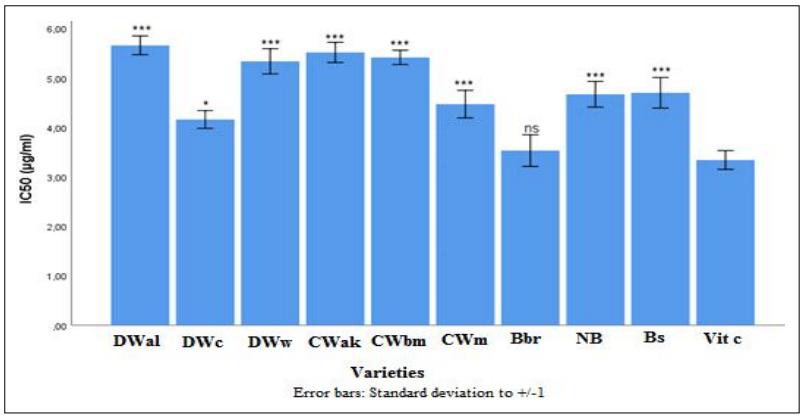


Fig 5: IC_{50} values of nine cereal varieties versus the ascorbic acid reference.

Table 3: The different identified phenolic compounds.

Peak. N°	Compound name	Retention time (min)
01	pholoroglucinol	2.4
02	Gallic acid	5.2
03	Protocatechuic acid	11.6
04	2.5 dihydrobenzoic acid	16.3
05	Vanillic acid	17.8
06	Syringic acid	19.8
07	Catechein	21.3
08	P.Coumarice	23.6
09	Rutin	24.6
10	Ferulic acid	25.9
11	Salicylic acid	26.7
12	Benzoic acid	29.9
13	O.Coumarice	30.6
14	Quercetin	33.2
15	Kaempferol	43.7

leaves. In general, our data indicate that phenols and flavonoids in cereals are major sources of natural antioxidants, followed by condensed tannins. Thus, the antioxidant capacity of cereal leaves seems to be largely influenced by the contents of flavonoids, tannins and total polyphenols (Fig 8).

According to the results of CAH (Fig 7 and 8), the tested varieties are divided into three different groups. The first group includes varieties poor in total polyphenols, flavonoids, tannins and even have the lowest percentages of inhibition, this is the case of the varieties Ain lahma (durum wheat al), Akhamokh (Common wheat ak) and Boumerzoug (Common wheat bm). The second group includes varieties that generally have average levels of total polyphenols, flavonoids, tannins and percentage of inhibition such as Massine (Common wheat m), Saida (Barley s), Cirta (Durum wheat c), Chaiir Ennabi (Naked Barley) and Wahbi (Durum Wheat w). The last group is characterized by a richness in total polyphenols, flavonoids, tannins and has a high

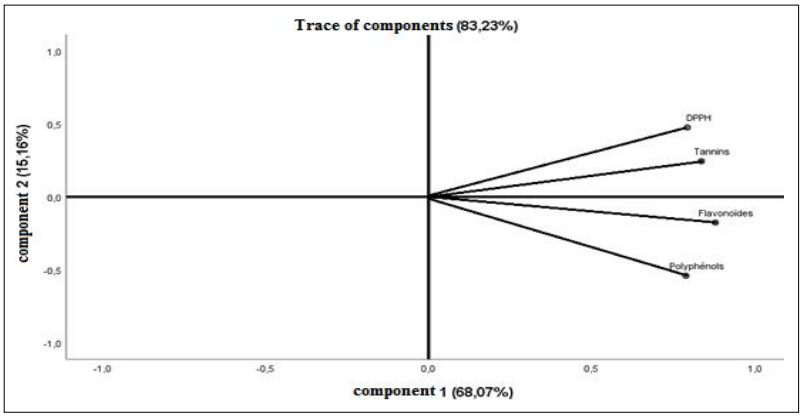


Fig 6: ACP of antioxidant activity (DPPH), contents of total polyphenols, flavonoids and tannins within cereal varieties.

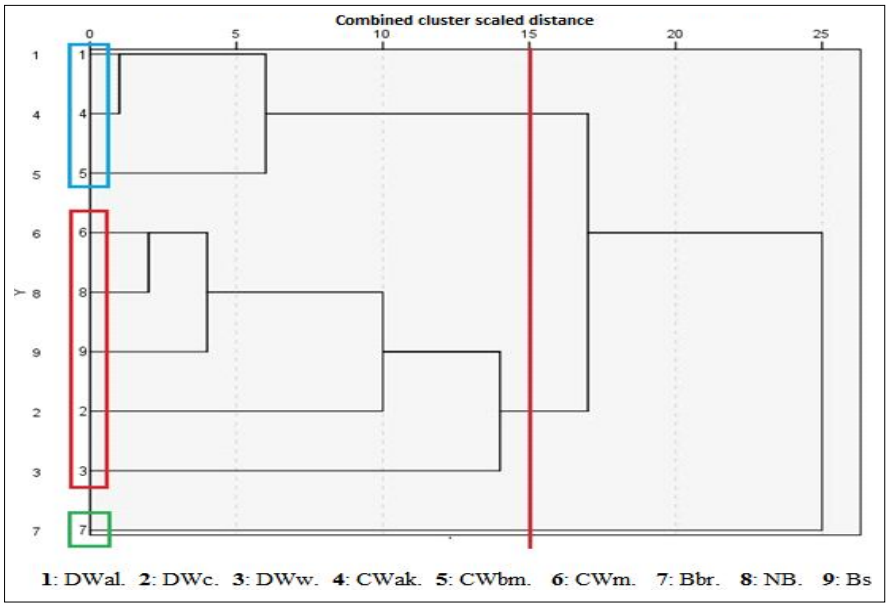


Fig 7: Dendrogram using the average distance between groups of varieties according to the CAH.

percentage of inhibition, this is the case of a single variety which is Barbarous (Barley br) (Fig 8).

The following chromatograms obtained by HPLC analysis of the phenolic extracts of the nine studied varieties. (Fig 9).

Based on HPLC analysis, several types of phenolic compounds were identified in the phenolic extracts of different varieties of studied cereals. We were able to identify 15 compounds (Table 3).

The major phenolic compounds identified in durum wheat species are protocatechuic acid, benzoic acid, pholoroglucinol, vanillic acid. And the minor phenolic compounds found are: catechin, p. coumaric, kaempferol, o. coumaric.

The major phenolic compounds identified in common wheat species are 2.5 dihydrobenzoic acid, pholoroglucinol, vanillic acid, gallic acid. And the minor phenolic compounds found are Ferulic Acid, P.Coumaric Acid, Catecheine, Rutin. The major phenolic compounds identified in barley species are pholoroglucinol, vanillic acid, gallic acid. And the minor phenolic compounds found are syringic acid, catechrin, p.coumaric acid, kaempferol.

The various studies carried out by several authors have shown that phenolic acids as strong antioxidants are present in barley (Adom and Liu 2002). Hydroxybenzoic acid is the major compound in cereals, with vanilic acid, protocatechuic acid, followed by gallic acid.

From the comparison of the retention times of our different peaks eluted with quercetin, we can say that the latter is absent in our different analyzed extracts. However, other research has identified quercetin in cereal leaves during fluorescence HPLC analysis, (Vasconcelos *et al*. 2013). The different peaks in the different chromatographic profiles can be classified into three phenolic groups which are: derivatives of flavones (Catecheine, rutin) those of cinnamic acids (ferulic acid, P. coumaric acid, O. coumaric acid) and derivatives benzoic acids (protocatechuic acid, benzoic acid, vanillic acid, gallic acid; these seem to be the majority).

For barley, methanolic extracts also contain compounds from the flavone and flavonol family and even phenolic acids. The main molecules that have been identified in barley are: keamphérol, keamphérol glycosyl, pholoroglucinol, vanillic

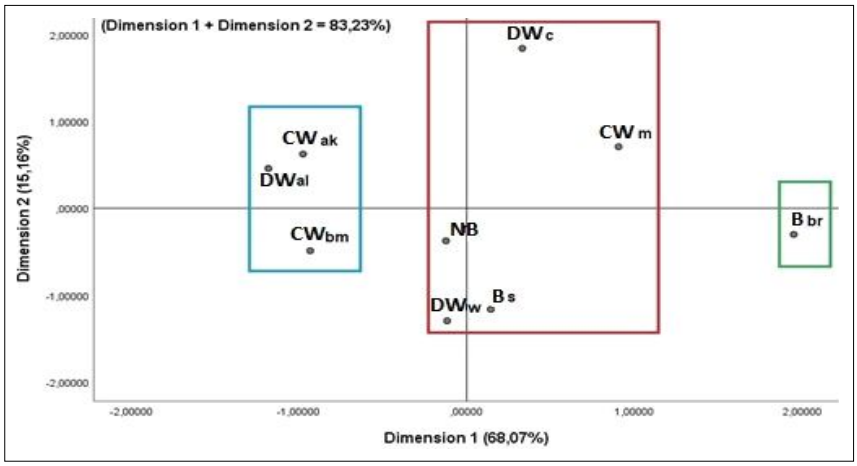
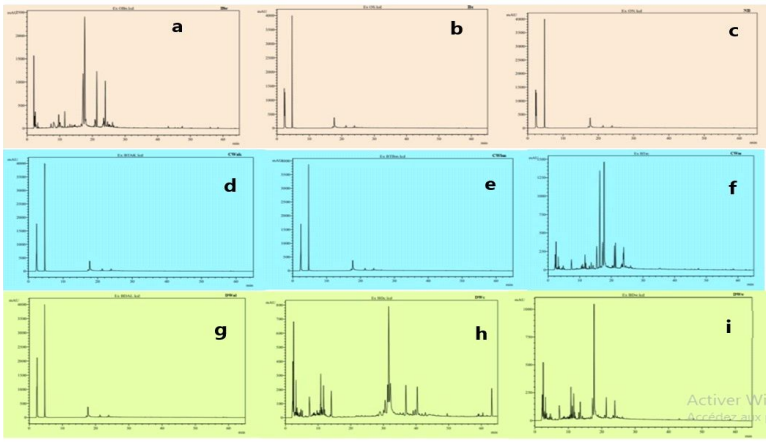


Fig 8: Distribution of varieties into three different groups according to Ascending Hierarchical Classification (AHC).



a): Bbr, b): Bs, c): NB, d): CWak, e): CWbm, f): CWm, g): DWal, h): DWc, i): DWw.

Fig 9: HPLC chromatogram of the methanolic extract of the different varieties.

acid. These are for the most part glycosylflavonols contrary to the results obtained by Harborne (1967) affirming that the glycosylflavones characterize the leaves of three cereals: barley which contains Lutonarin 3′-methyl ether, oats which contain 8-c (arabinosylglycosyl)-apigenin and wheat: Titicoccum dicoccum contains: 6-c- (rhamnosylglycosyl)-luteolin.

CONCLUSION

According to the results of the ACP, the varieties rich in tannins and flavonoids have a significant antioxidant activity than a variety rich only in total polyphenols. The Barbarous variety (Barley br) is the only variety that has an IC50 that does not differ statistically from the ascorbic acid reference with a high PI (76.49%) and therefore significant antioxidant activity and high levels of polyphenols, flavonoids and even in tannins.the Cirta variety (Durum wheat c) despite having generally average contents of total polyphenols, flavonoids and tannins but it has the highest percentage of inhibition among all the varieties (76.71%).The present study revealed that cereal leaf extracts demonstrated high phenol content and potent antioxidant activity, achieved by free radical scavenging and reducing potency tests. Barley variety Barbarous had the highest antioxidant activities and the highest contents of total phenols, total flavonoids and condensed tannins.

The results obtained show that the phytochemical composition varies greatly between the different analyzed cereal samples. These differences can be explained by genetic control, since all varieties were grown under the same environmental conditions. The large variations observed in this study may be important for the optimal use of these varieties for food, fodder and industrial purposes.

Qualitative studies based on HPLC of these compounds allow us to visualize phynolic traces of our extracts, this analysis showed that our extracts mainly contain flavonoids of the flavone and flavonol type, potentially interesting for their biological properties., therefore these cereals contain molecules which are considered first-class antioxidant agents and can be used for therapeutic applications, knowing that antioxidants contribute very effectively to prevent various diseases such as Alzheimer's disease and cancer, which are major problems in public health. Knowing that our country has an immense biodiversity of which each plant is characterized by a fairly large reservoir of secondary metabolites with therapeutic, pharmacological and nutritional characteristics such as wheat leaves and barley leaves which require to be explored by research.

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Conflict of interest: None.

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