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THEME

Determination the antioxidante activity of seeds, leaves and shelled panicles of different varieties of pearl millet (*Pennisetum glaucum* (L). Br) cultivated from Bordj bou Arreridj region.

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Dedication

To my family and friends

Messoudi sarah, hadjadj nour alhouda and djoual fatiha

And all the people that helped me and stand by my side through all this
years.

I dedicate this modest work.

List of abbreviations

(AEAC):	ascorbic acid equivalent antioxidant capacity
(ANOVA):	analysis of variance.
(CE): matter	mg catechine equivalent (CE) per 1g of plant
(DPPH °):	(1, 1-diphenyl-2-picrylhydrazyl)
(FeCl₃):	iron (III)chloride hexahydrate .
(FRAP):	(Ferric Reducing antioxidant Power)
(M3):	millet 3
(M7):	millet 7
(M11):	millet 11
(mg GAE /g plant matter) :	Gallic acid equivalent per 1 g of plant matter
Nc	not calculated
(SD):	standard deviations.
(TCA):	trichloric acid.
(TPC):	total phenolic content.
Me	Methanol.
Me/H	Hydrolyzed methanol.
Ac	Acetone.
Ac/H	Hydrolyzed acetone.

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Introduction

I.Introduction

Because of today's chase for healthy products, the production and purification of vegetable extracts is an area of interests to the industry and academia. Recently, researchers have shown a proof for the role of antioxidants of plant origin on scavenging of free-radical, this property could have great importance as therapeutic agents in several diseases caused due to oxidative stress such as heart diseases and cancers. However, plant constituents and phytochemical compounds were to be found effective as radical scavengers and inhibitors of lipid peroxidation. **(Mahantesh, et al.(2012))**

The cellular functions and normal respiration produce a free radical. Under normal physiological conditions, approximately 2% of oxygen used up by the human body during respiration is transformed into superoxide anion free radical, with a negative charge ($O\cdot^{2-}$) **(Kunwar, A., & Priyadarsini, K. I. (2011))** . This oxygen environment is unfriendly to the living organisms. To survive, this organism produces a water- and lipid-soluble antioxidants that can neutralize these highly reactive free radicals. If the body's antioxidant mechanism does not operate optimally, excess free radicals can damage various biomolecules, including lipids, proteins, carbohydrates, and nucleic acids. **(Rice-Evans, et al(1995, November))**

A variety of antioxidants is found in dietary sources like fruits and vegetables which were known as phytochemicals compounds. These plant constituents are classified as primary or secondary components, depending on their role in plant metabolism. Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophylls etc. Secondary constituents are the remaining plant chemicals such as alkaloids (derived from amino acids), terpenes (a group of lipids) and phenolics **(Krishnaiah Det al. (2007))**.

They are present in all parts of higher plants (stems, leaves, flowers, pollen, fruit, seeds and wood) and are involved in many physiological processes such as cell growth, rhizogenesis, germination of the seeds or fruit maturation. The most represented are the anthocyanins, flavonoids, and tannins **(Boizot.N, Charpentier, J.P. (2006))**.

Several reports have shown that millet **(Pathak,Srivastava, & Grover, 2000)** is inexpensive and nutritionally comparable or even superior to major cereals. Grain millet is also important food cereals in many parts of Africa, Asia and the semi-arid tropics world wide. In Africa, India and China, grain millet comes fourth among cereals for human consumption, superseded only by rice, wheat and sorghum. In Northern and Eastern Europe, rye is a traditional cereal

that is generally used as whole meal flour in both soft and crisp breads. **(Raj Kumar Salar a,*,et all (2016)).**

The millet grain contains about 65% to 72% carbohydrates, a high proportion of which is non-starchy polysaccharides and dietary fiber. The fat from millet contains a high proportion of essential fatty acids, and the protein has a balanced essential amino acid profile **(S. Hegde, et all .(2005)).** Table01

Millet is an important source of some minerals, particularly iron, zinc, and calcium **(table01). (Mahalingam Govindaraj, et all (2018))**

Table 1: Nutrient composition of finger millet compared to rice and wheat (All values are per 100 g of edible portion)

Name	Protein (g)	Carbohydrate (g)	Fat (g)	Crude fiber (g)	Total dietary fiber (g)	Energy (J)	Phytin P (mg)	Mineral matter (g)	Ca (mg)	Fe (mg)	Zn (mg)	P (mg)
Finger millett (Eleusine coracana)	7.3	72.0	1.3	3.6	19.1	1372.35	209	2.7	344	3.9	2.3	283
Raw milletted rice (Oryza sativa)	6.8	78.2	0.5	0.2	NA	1443.48	83	0.6	10	0.7	1.3	160
Whole wheat (Triticum aestivum)	11.8	71.2	1.5	1.2	11.4	1447.66	238	1.5	41	5.3	2.7	306

The polyphenolic compounds of foxtail millet seeds are mainly composed of diversity of flavonoids and phenolic acids. It was reported by Pradeep and Sreerama that caffeic acid, ferulic acid and sinapic acid were the main phenolic acids and kaempferol and luteolin were the main flavonoids in free phenolic extracts, and p-coumaric acid and ferulic acid were abundant in the bound phenolic extracts of foxtail milletlet from India. Apart from the common reported phenolic acids, including p-coumaric acid, syringic acid and ferulic acid, nine hydroxycinnamic acid spermidines and kaempferol-C-glycosides and apigenin-C-glycosides were found in the free phenolic extracts of dehulled foxtail millet from China in our previous research. **(A. Chandrashekar, et al.(2006)).**

Table 2: Phenolic acid composition of some millets (Linda Dykes, Lloyd W. Rooney,(2006).

	<i>Finger</i>	<i>Pearl</i>	<i>Proso</i>	<i>Teff</i>	<i>Fonio</i>	<i>Foxtail</i>
a) Phenolic assaya						
Folin/Ciocalteu	0.55-0.59	0.19-0.33	0.05-0.10	0.09-0.15	0.14	0.12
Vanillin/HCl	0.17-0.32	0.05	0	0	0	0
b) Phenolic acidsc						
Protocatechuic	23.1	11.8	-d	25.5	-d	-d
Gentisic	61.5	96.3	-d	15	-d	21.5
p-Hydroxybenzoic	8.9	22	-d	-d	-d	14.6
Vanillin	15.2	16.3	-d	54.8	-d	87.1
Caffeic	16.6	21.3	-d	3.9	-d	10.6
Syringic	7.7	17.3	-d	14.9	-d	93.6
Coumaric	56.9	268.2	-d	36.9	-d	2133.7
Farulic	387	679.7	-d	285.9	-d	765.8
Cinnamic	35.1	345.3	-d	46	-d	781.7

a : Values are expressed on mg/100 mg catechin equivalents, dry weight basis. All millets have phenols but only finger millet has condensed tannins.

Pearl millet gives a reading with the vanillin assay, but does not contain condensed tannins.

b :µg phenolic acid/mg samples, as is moisture basis.

d : Value not available.

Finger millet is a potent source of antioxidants and has potent radical scavenging activity that is higher than that of wheat and rice; these results corresponded to their phenolic content (**Raj Kumar Salar a,***, **Sukhvinder Singh Purewal a** , **Manpreet Singh Bhatti(2016)**). The brown or red variety of finger millet, which is commonly available, had higher activity (94%) than the white variety (4%) using the DPPH Kodo millet quenched DPPH by nearly 70% higher than other millets (15–53%); white millet varieties had lower activity (**Linda Dykes, Lloyd W. Rooney,(2006)**). Luteolin, a flavone present in sorghum and millets, has been reported to have antioxidant, anti-inflammatory, cancer-preventive, anti-arrhythmic properties Tricin has been reported to have anti-tumor and antileukemic properties (**Linda Dykes, Lloyd W. Rooney,(2006)**).

The millet can also grow and give higher and more stable grain yields in regions characterized by low rainfall or drought, high temperature and low soil fertility. The environment and climate in the south of Algeria are characterized by such conditions, and thus adaptation of this cereal grain in Algeria is of great interest. For this, the Algerian state has recently paid attention to this cultivation.

In this scope, our study of Algerian millet is based on two criteria: the first is to promote the future development of the industrial production of this cereal in Algeria, which needs to evaluate their nutritional quality and potential uses. The second criterion is based on the fact that the millet contains constituents that have demonstrated health benefits for humans. **(Linda Dykes, Lloyd W. Rooney,(2006)).**

Material and method

II. Materials and methods

II.2. Plant collection and identification

II.2.1 Plant material

The pearl millet plant is an annual grass that grows to a height of 1-2 meters (3-6 feet). It has a robust, upright stem and long, slender leaves that are typically green or bluish-gray in color. The leaves are arranged in a spiral pattern around the stem and are typically 30-50 centimeters (12-20 inches) long and 1-2 centimeters (0.4-0.8 inches) wide.

The leaves are attached to the stem by a sheath, which is a tubular structure that encloses the base of the leaf. The sheath is typically open at the top and closed at the bottom, and it serves to protect the base of the leaf and help the plant retain moisture. The plant produces clusters of small, inconspicuous flowers that are arranged in a terminal inflorescence, or flowering structure. (Gougue Fatna.(2010)).



Figure 1: pearl millet plant.

II .2.2 Materials

Balance (Pioneer (DHAUS)), UV-visible spectrophotometry (SP-3000nano (OPTIMA)), ultrasound (P SELECTA), water bath, test tubes.

II 2.3 Sample preparation

Seeds, leaves, and shelled panicles samples were manually separated and then were air-dried in shadow at room temperature and reduced to fine powder.



Figure 2: type of samples shelled panicles¹, grains², leaves³ of pearl millet.

II.2.4 Chemicals and reagents

methanol, acetone, folin-ciocalteu reagent, Gallic acid, vanillin, catechine, DPPH(1.1-Diphenyle-2-picryldrazyl), hydrochloric acid (HCl), ascorbic acid these products were obtained from (sigma-aldrich, Inc. (chemie GmbH, Steinheim, Germany)). whereas Potassium dihydrogen phosphate (KH_2PO_4) (Fluka chemika), iron (III)chloride hexahydrate (FeCl_3) (Vwr Prolabo (Belgium product)) di-potassium Hydrogen phosphate anhydrous (K_2HPO_4) (Panreac ITW companies). PART DUMAS filter paper from (lab materials, France).

II.3 Phenolic compounds extraction

II.3.1 Extraction method

Ultrasound Assisted Extraction

A fine dried powder of samples (1 g) was dissolved with the extracting solvent (methanol, (methanol:water (8/2; v/v)), acetone, (acetone:water (7/3; v/v)), placed in a ultrasonic bath during 45min at 30°C , The extracts were pooled, filtered and evaporated and made up to 5ml with methanol and stored at 4°C till use. Fig03



Figure 3: Ultrasound extraction and filtration.

II.4 Phenolic extracts quantification

II.4.1 Phenol content quantification

Principle

The Folin-Ciocalteu assay is the most widely used method for the estimation of total phenolic content (TPC) in extracts fruits, vegetables, grains, and other foods. The FC reagent consists of mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMO_{12}O_{40}$) which, was reduced during oxidation of phenols to produce a mixture of blue molybdenum (Mo_8O_{23}) and tungsten (W_8O_{23}) oxides (λ_{max} 765 nm). (Ben Haoua, K. (2019))

Protocol

Total phenolics content was determined with the Folin-Ciocalteu reagent according to a method described by Singleton-Ross (Singleton et Ross en 1965). Briefly, 100 μ l of the extract was added to 0.5 ml of Folin-Ciocalteu (10%) reagent. Then 2 ml of 2% Na_2CO_3 solution. The reaction mixture was kept in the dark for 30 min, and its absorbance was measured at 760 nm against a blank.

Gallic acid and tannic acid were used as a references standards. Total phenolic content was expressed as mg Gallic acid equivalent (GAE) per 1 g of dry matter (mg GAE /g plant matter) and as mg tannic acid equivalent (TAE) per 1 g of dry matter (mg TAE/g plant matter)

The total phenolic concentration could be calculated according to Gallic/Tannic acid concentration by the following equation:

$$(\text{mgGAE/g}) = (\text{mgTAE/g}) = \left(\frac{\text{Abs}_{\text{sample}} - \text{Abs}_c}{B} \right) \times \left(\frac{V \times D}{W_{\text{sample}}} \right)$$

Where:

$\text{Abs}_{\text{sample}}$: Extract absorbance.

Abs_c : Absorbance without folin-ciocalteu (discoloration).

B: slop of gallic acid standard curve.

V: total extract volume.

D: dilution factor of the extract.

W_{sample} : Sample initial weight used for extraction (g)

II.4.2 Condensed tannin quantification

Principle

The dosage of condensed tannins was carried out by the vanilin hydrochloride method which is based on the condensation of polyphenolic compounds. (Price, et al (1978)).

Its principle is based on the fixing of the aldehyde group of vanillin on carbon 6 of cycle A of the catechin or condensed tannin (figure 06) to form a red chromophore complex that will be measured at 500nm. (Mrabti,H.N(2018)).

Protocol

(1%) vanillin in methanol was prepared then mixed with HCl (8%) (50ml/50ml v/v), (solution (A)), then it was conserved at 30°C before the quantification , 100µl of extract was added to 1000µl of vanillin reagent to quantify the condensed tannins , the blank was prepared by replacing the reactive by distilled water (H₂O), the tubes was conserved at 30°C during 20 min , the absorbance was read at 500nm . the catechine was used as standard , the results was expressed as mg catechine equivalent (CE) per 1g (CE/g) of plant matter . (Mrabti,H.N(2018))

The condensed tannin concentration could be calculated according to Catechine concentration by the following equation:

$$(mgEC / g) = \left(\frac{Abs_{sampl} + b}{B} \right) \times \left(\frac{V \times D}{W_{sample}} \right)$$

Where:

Abs_{sample} : Extract absorbance.

B: slop of catechine standard curve.

b: y-intercept.

V: total extract volume.

D: dilution factor of the extract.

W_{sample} : Sample initial weight used for extraction (g)

II.5 Antioxidant activity of extracts

II.5.1 DPPH assay

Principle

The 1,1-diphenyl 1-2-picrylhydrazyl (DPPH•) - which is also known as α,α -diphenyl- β - picrylhydrazyl, 2,2-diphenyl -1- picrylhydrazyl or 2, 2-Diphenyl -1-(2,4,6-Trinitrophenyl hydroxyl) assay originally described by Blois (1958) (Blois, M.S. (1958)) was designed to take advantage of a common electron spin resonance reagent, a stable free radical with an odd, unpaired valence electron to study antioxidant activity . With its odd electron, DPPH• (Sharma, O.P. and Bhat, T.K. (2009)); can be stabilized by accepting an electron or hydrogen radical from an antioxidant molecule such as a Gallic acid as a reducing agent. DPPH• is known for its deep violet color and the strong absorbance at 517nm when dissolved in methanol ((Blois, M.S. (1958)), this absorbance is decreased with the decolorization of DPPH• which accompanies the pairing of the lone electron.

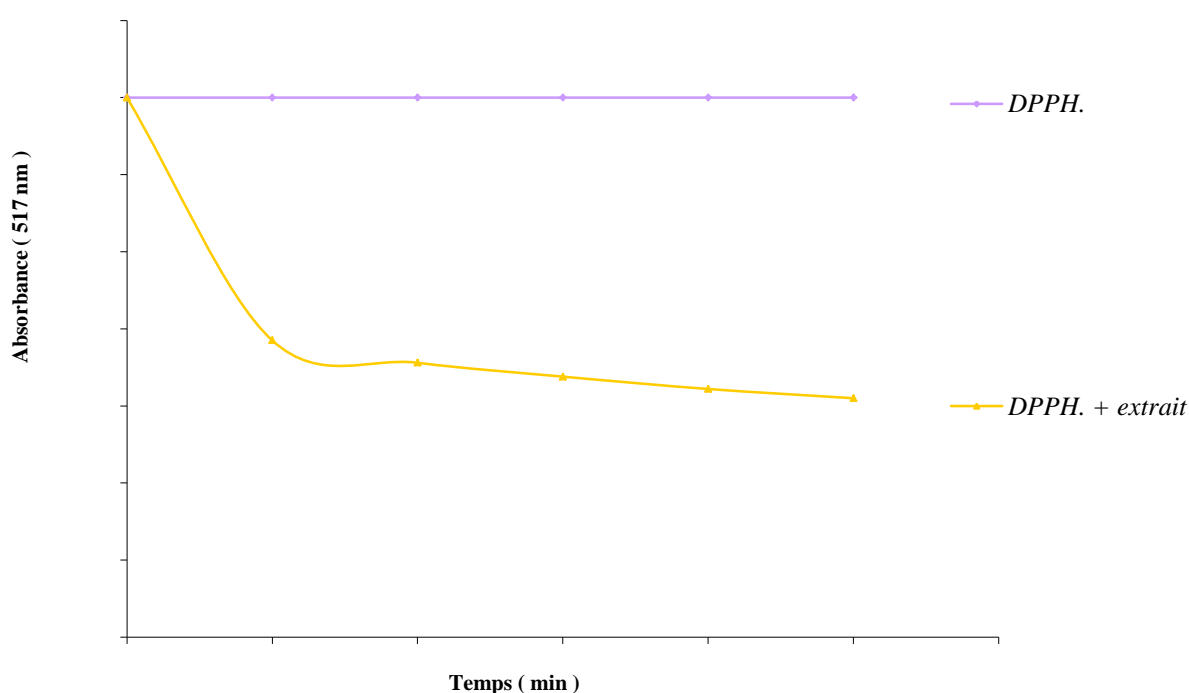


Figure 4: curve shows the difference between Absorbance of DPPH solution without extracts and the absorbance with extracts.

Protocol

100 uL of diluted extract and 1 mL of DPPH were mixed. After 30 min of incubation at room temperature, the absorbance was read against a blank at 517 nm (SP-3000nano (OPTIMA)). Inhibition of DPPH free radical in percent (I%) was calculated as follows:

$$I \% = \frac{A - A_0}{A_0}$$

Where:

I% : Inhibition percentage (%)

A : Absorbance of DPPH solution without extracts

A₀ : Absorbance of DPPH solution containing extracts after 30 min of reaction time.

Where a blank is the absorbance of the control reaction (containing all reagents except the test compound) and A sample is the absorbance of the test compound. Extract concentration providing 50% inhibition (EC50) was calculated from the graph plotting inhibition percentage against extract concentration. All tests were carried out in triplicate or more depending on the concentration of each extract . The ascorbic acid was used as positive control.

II.5.2 FRAP assay

Principle

The FRAP method is based on reducing the ferric iron ion (Fe^{3+}) to ferrous (Fe^{2+}) ion. This method evaluate the reduction power of the compound

The presence of (AH) reducer in the plant's extract provoke the reduction of the ferric cyanide complex (Fe^{3+}) into (Fe^{2+}) form. as a result , (Fe^{2+})ions could be evaluated and measured mean while observing the augmentation of density and cyan blue coloration in the interactive field at 700nm .

In fact, the ($FeCl_3/k_3Fe(CN)_6$) system offers the sensibility to the method in order determines the (semi quantitative) concentrations of the anti-oxidants that participate in the redox reaction. (**Benzie et all (1996)**).

Protocol:

This FRAP method is cited by Oyaizu,(1986). (**Oyaizu, M. (1986)**). It's used to determine the reduction power, 25 ul of extract and 500ul of (A) and (B) was mixed (where (A) K_2HPO_4 and (B) KH_2PO_4) and 500ul of ($FeCl_3/k_3Fe(CN)_6$) the tubes was conserved at 50°C during 20min then 500 ul of TCA and 100 ul of $FeCl_3$ (0.1%) was added . after that absorbance was read at 700nm.

Calibration curve: vitamin c

Starting by standard solution of vitamin C (0.04g/l), a range of standard concentrations was prepared it varied from 0.05(g/l) to 8(g/l) .Next the same protocol was followed to quantify the samples.

Ferric Reducing antioxidant Power could be expressed according to vitamin C.

II.6 Statistics analysis

All analyses of phytochemical and anti-oxidant activity were performed in triplicate and the data were reported as means \pm standard deviations (SD). The means were compared using the one-way analysis of variance (ANOVA) followed by Tukey's multiple range test. The Pearson correlation coefficients were calculated to determine relationships among the observed traits, using adjusted means. All analyses were performed using SPSS v.17 software. Differences were considered significant at $P < 0.05$. (Appendix 1)

Results and discussion

III. Results and discussion

The general objective of this study is to extract and quantify the phenolic compounds present in different parts (grains, shelled panicles, leaves) of three cultivars of pearl millet plants grown in Bordj Bou Arreridj, using four different extraction systems and evaluate their antioxidant activities according to the DPPH free radical scavenging method and the FRAP iron reduction method.

III.1 Extraction yield

The values of the yields in percentage of the crude extracts of our samples of each fraction (acetone, methanol, acetone:water (70/30) and methanol:water (80/20)) of the seeds, leaves and shelled panicles of three varieties of millet are presented in the following table

Table 3 : The values of the yields in percentage of the crude extracts.

System/organ	Millet3			Millet 7			Millet 11		
	Seeds	Leafs	shelled panicles	seeds	Leafs	shelled panicles	seeds	Leafs	shelled panicles
Acet	5.04	3.5	5.04	0.63	6.4	4.24	Nc	9.26	2.56
MetOH	3.26	5.6	3.2	8.2	7.18	4.92	0.105	0.725	3.1
Acet/H ₂ O(70/30)	Nc	Nc	6.8	2.33	Nc	Nc	Nc	Nc	2.68
MetOH/H ₂ O(80/20)	Nc	Nc	2.2	4.6	3.7	1.6	4.9	0.92	8.33

- According to these values, it can be seen that the variation in yields is recorded according to the varieties, the different parts of millet (grains, shelled panicles, leaves) and the extraction system.
- The percentages of the yield vary between (0.63-9.26%) for the acetone extracts and (0.105-7.18%) for those of methanol. For the system acetone: water and methanol: water, the crude extract content varied between (2.33-6.8%) and (0.92-8.33%) respectively.
- Overall the yields of the acetone extracts are the highest than the other extraction systems.
- If we are interested in the different parts of millet, we will deduce that the leaves have the highest yield (9.26%) followed by the panicle shelled with a percentage of panicle shelled (8.33%) both in M11 which reflects the accumulation of polar compounds in and Leaves in this variety .fig05

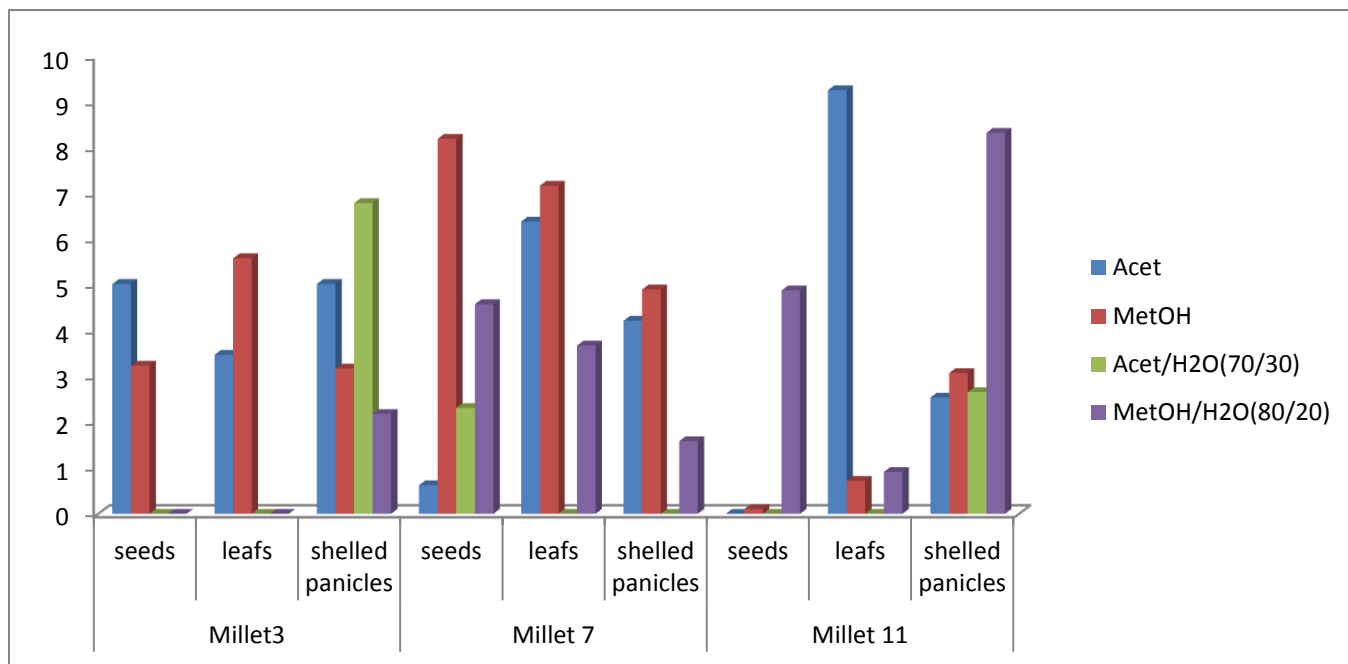


Figure 5: The values of the yields in percentage of the crude extracts.

III. 2. Phytochemical analysis

III.2.1 Total phenolic content (TPC)

Total phenolic content in seeds, leaves and shelled panicles extract was expressed in terms of Gallic acid equivalent (GAE) per gram fig 06.

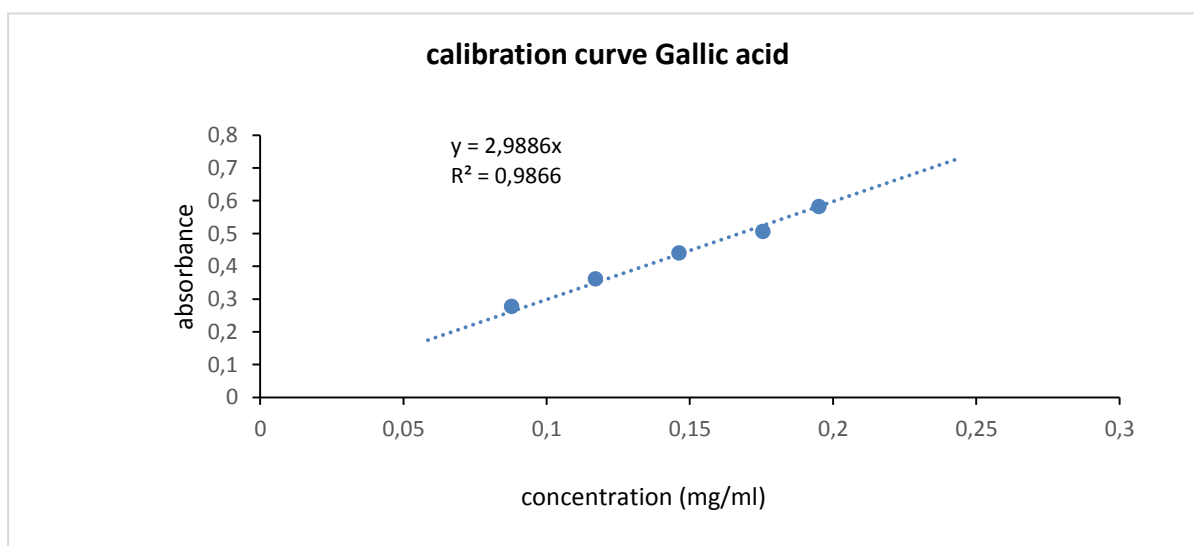


Figure 6: calibration curve used in phenolic quantification Gallic acid.

Total polyphenols were quantified in the extracts in order to compare the four solvents and the three organs of three varieties of millet (Table04)

Table 4:Total phenols content of millet (mgGAE/g extract).

System/organ	Millet3			Millet 7			Millet 11		
	seeds	leaves	shelled panicles	Seeds	Leaves	shelled panicles	seeds	Leaves	shelled panicles
Acet	0.34 ± 0,07	0.27 ± 0,06	0.48 ± 0,12	0.24 ± 0,04	0.46 ± 0,20	0.23 ± 0,03	1.53 ± 0,05	0.75 ± 0,02	0.11 ± 0,01
MetOH	1.01 ± 0,82	2.48 ± 0,03	1.3 ± 0,02	0.69 ± 0,05	1.27 ± 0,11	0.72 ± 0,10	1.15 ± 0,03	2.42 ± 0,10	0.71 ± 0,27
Acet/H ₂ O(70/30)	0.62 ± 0,18	1.48 ± 0,13	1.53 ± 0,03	0.81 ± 0,04	1.8 ± 0,01	0.15 ± 0,01	0.23 ± 0,05	2.72 ± 0,14	0.75 ± 0,05
MetOH/H ₂ O(80/20)	0.93 ± 0,14	1.47 ± 0,04	0.71 ± 0,01	0.95 ± 0,07	0.75 ± 0,01	0.12 ± 0,05	0.71 ± 0,03	2.52 ± 0,10	0.46 ± 0,07

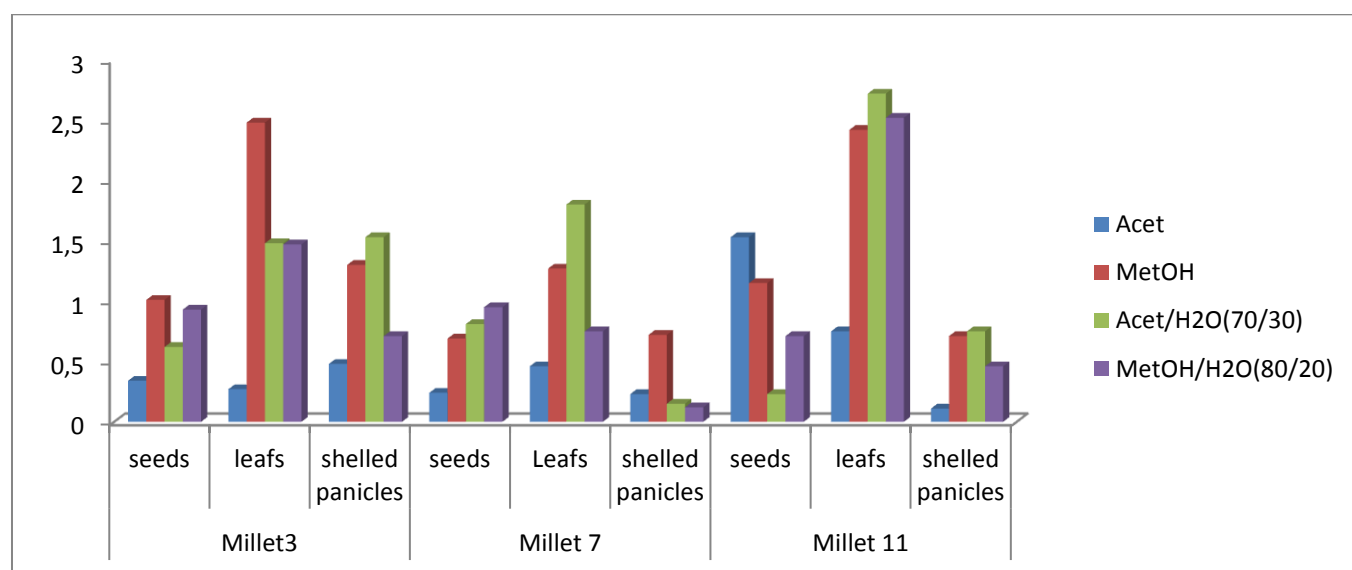


Figure 7: Comparison of phenol content of extracts (mgGAE/g) from different parts of millet.

- the amount of dry residues in the table 01 is higher than the amount of phenolic compounds (table04), it can be explained that the solvents used do not only give polyphenols but other non-phenolic substances such as sugars, proteins, pigments.
- As a conclusion, the results recorded in the table (04) and fig12, it can be observed that the higher content of phenolic compounds is found in the leaves with Acet/H₂O(70/30) system In M11.

Actually, the total content of phenolics is controlled by many parameters and conditions, one of them is the environmental factors in which plant grows such as: the season and date of collecting, soil composition, climate, temperature, light, humidity, salt and water stress. Also, the quantity and quality of the compound are greatly affected by the plant age and its stage of growth. Drying and extraction methods have an effective role in the total contents of phenolic in the extracts (CHENGUEL Aouatef ,.(2019))

III.2.2 tannins content (TPC)

The tannins compounds were determined using the method of vanillin/HCl. where was catechine used as a reference standard to estimate tannins content.fig08

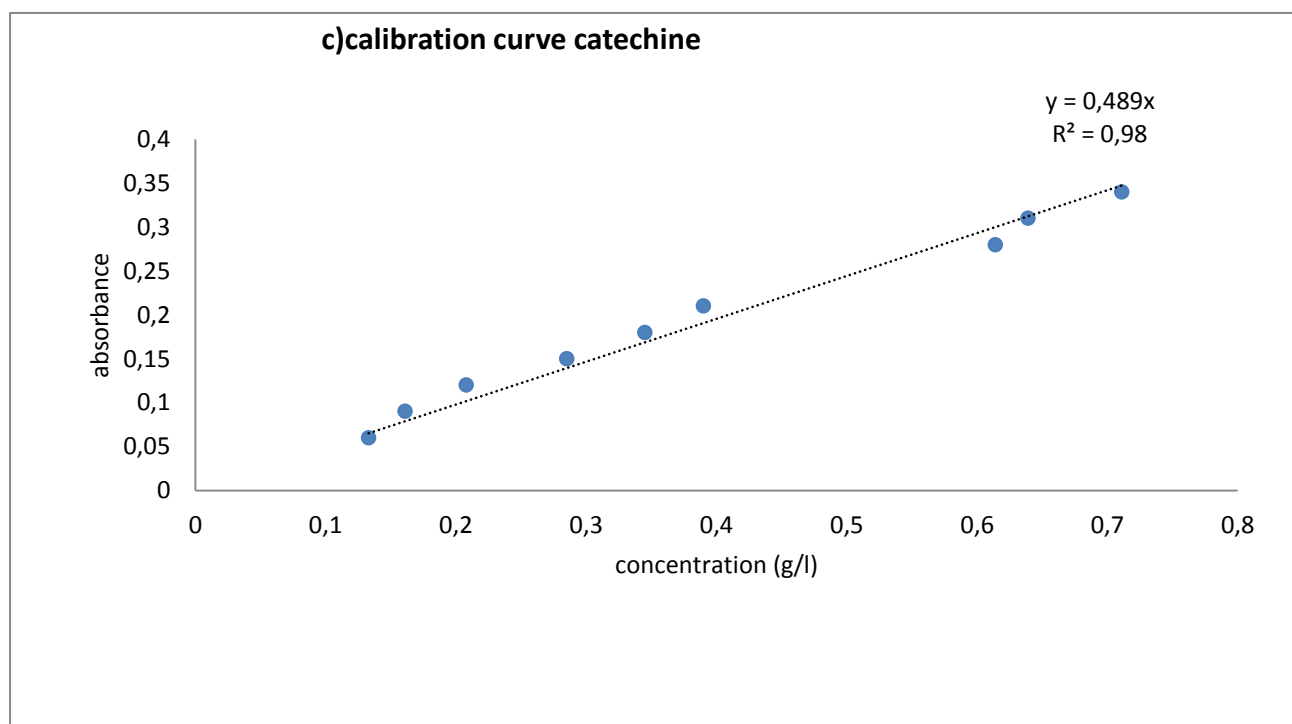


Figure 8: calibration curves used in tannin quantification with catechine.

Tannins were quantified in the extracts in order to compare the four solvents and the three organs of three varieties of millet (Table 6).

Table 5:Table of tannin content of millet (mgEC/ g plant matter).

System/organ	Millet3			Millet 7			Millet 11		
	seeds	Leaves	shelled panicles	seeds	Leaves	shelled panicles	seeds	leaves	shelled panicles
Acet	10.39 ± 0.01	0.68 ± 0.03	8.58 ± 0.14	nd	1.97 ± 0.22	6.79 ± 0.88	Nc	5.16 ± 0.07	1.40 ± 0.16
MetOH	11.61 ± 0.35	4.09 ± 0.04	9.81 ± 0.02	8.91 ± 0.02	0.94 ± 0.3	5.54 ± 0.14	8.76 ± 0.52	2.71 ± 0.06	1.26 ± 0.05
Acet/H ₂ O(70/30)	Nc	1.00 ± 0.02	10.66 ± 1.35	0.39 ± 0.02	6.91 ± 0.09	0.03 ± 0.01	0.10 ± 0.02	6.81 ± 0.04	0.41 ± 0.05
MetOH/H ₂ O(80/20)	0.39 ± 0.02	0.80 ± 0.08	4.77 ± 0.55	0.99 ± 0.01	0.59 ± 0.04	Nc	0.10 ± 0.01	2.12 ± 0.07	0.36 ± 0.05

if we compare the levels of tannins extracted by the four solvents, the results varied from (0.03 to 11.68 EC/g), the highest and the converge result was given by system (methanol) and (acetone/water: 70/30) with (11.61 EC/g) and (10.66 EC/g) respectively, followed by system (acetone 100%) and (methanol/water: 80/20 v/v) with (10.39 EC/g) (4.77 EC/g) respectively.

The tannin content is highest in the samples of M3 with the values 11.62 EC/g and 10.66 EC/g respectively while the lowest content tannin in M7 was the lowest with (0.03EC/g)

The contents of tannins in the extracts are higher than those of total phenols which are not reasonable (table 4 and 5). This difference may be due to the standard phenols used for the preparation of the calibration curves or even to the methods chosen for the assay.

The same high values of tannins were found by Prashant S. when he dosed the tannins of millet and sorghum (**Prashant S. Hegde, T.S. Chandra(2005)**).

III.3 Antioxidant activity

In this study, two different assays were used to evaluate antioxidant activities of millet (seeds, leaves and shelled panicles). Each of them employs a different mechanism and reaction to detect antioxidant compounds. We applied these two assays because there is no single assay, which will accurately reflect all antioxidants and radical sources in such an intricate system due to their complex composition also assert that there is no simple universal antioxidant assay by which antioxidant capacity can be measured accurately and quantitatively. (**NUUTILA A. M.,et all., 2003**), **PRIOR R.et all (2005)**)

III.3.1 DPPH radical scavenging activity assay

In this study, the DPPH method was selected to evaluate the antioxidant activity of plant extracts because it is one of the most effective methods for evaluating the concentration of radical-scavenging materials active by a chain-breaking mechanism. The DPPH radical is a stable free radical and the DPPH radical-scavenging activity was determined by the decrease in absorbance at 517 nm, due to reduction by the antioxidant (AH) or reaction with a radical species, (**Pitchaon Maisuthisakul a ,et all (2007)Assessment**)

The DPPH radical-scavenging capacity in the studies was reported after 30 min reaction time for each diluted plant extract. most of the plots of the plant extracts showed linear curves.

VEAC value, defined as the concentration of antioxidant required to qualify 1g of vitamin c scavenging ability , is a parameter widely used to measure antioxidant activity; the higher (VEAC) value corresponds to a higher antioxidant activity of the plant extract (**Pitchaon Maisuthisakul et all(2007)**)

The (VEAC) value of various plant extracts shown in Table (7) was determined based on the calibration curve of vitamin c fig09.

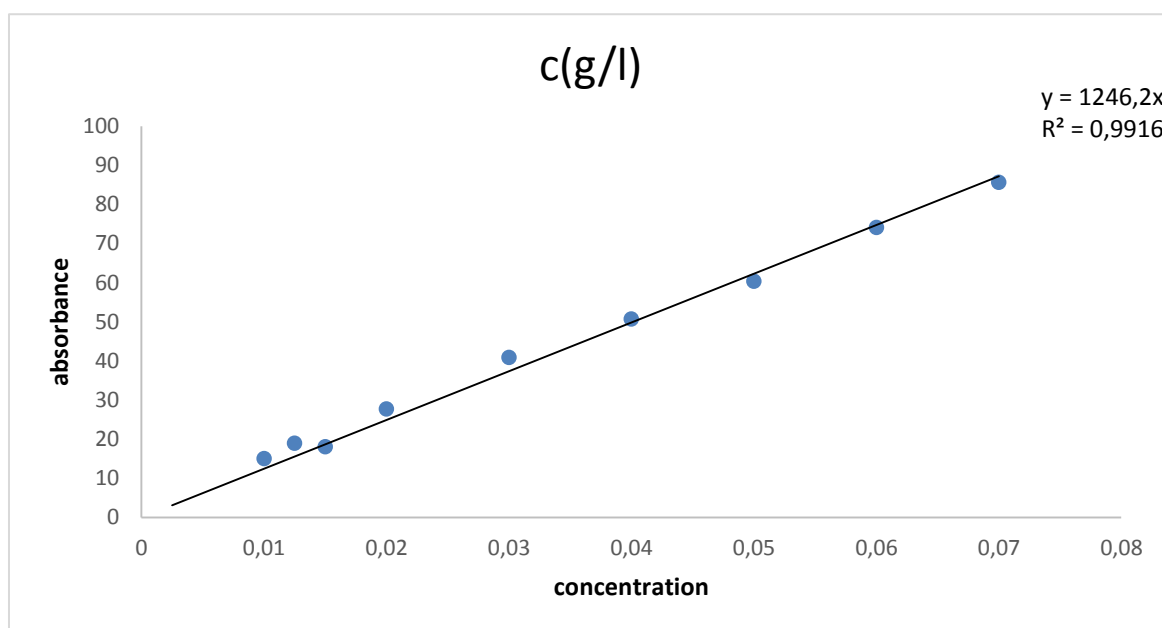


Figure 9: calibration curve ascorbic acid.

Table 6:The VEAC (DPPH assay) value of various plant extracts.

System/organ	Millet3			Millet 7			Millet 11		
	Seeds	Leaves	shelled panicles	Seeds	Leaves	shelled panicle	Seeds	Leaves	shelled panicle
Acet	10,39 ± 0,01	0,68 ± 0,43	8,85 ± 0,13	Nc	1,97 ± 0,05	6,79 ± 0,75	8,76 ± 0,01	5,16 ± 0,05	1,40 ± 0,03
MetOH	11,61 ± 0,44	4,092 ± 0,01	9,81 ± 0,25	8,92 ± 0,26	0,94 ± 0,76	5,54 ± 0,26	Nc	2,71 ± 0,05	1,26 ± 0,10
Acet/H2O(70/30)	Nc	1,09 ± 0,21	10,66 ± 0,18	0,39 ± 0,06	6,91 ± 0,08	0,037 ± 0,23	0,107 ± 0,01	6,81 ± 0,23	0,36 ± 0,26
MetOH/H2O(80/20)	0,39 ± 0,44	0,807 ± 0,38	4,77 ± 0,04	0,99 ± 0,63	0,59 ± 0,66	Nc	0,107 ± 0,16	2,12 ± 0,42	0,41 ± 0,32

The DPPH results according to vitamin C equivalent antioxidant capacity (VEAC) values varied between 0.037 and 11.61mg/ml .

methanol extracts had positive results with all the organs of different varieties but the leaves of M7. Table (06)

On one hand the seeds of M3 extracted by methanol contains average amount of phenolics and high amount of tannins these seeds found to have high DPPH scavenging ability according to ascorbic acid (11.61mg/ml), compared to those obtained by other solvent extracts.

On the other hand, the second high hydroxyl radical inhibition was observed in shelled panicles of M3 with system aqueous acetone 70/30 (10.66mg/ml) also this extract possess high content of phenols.

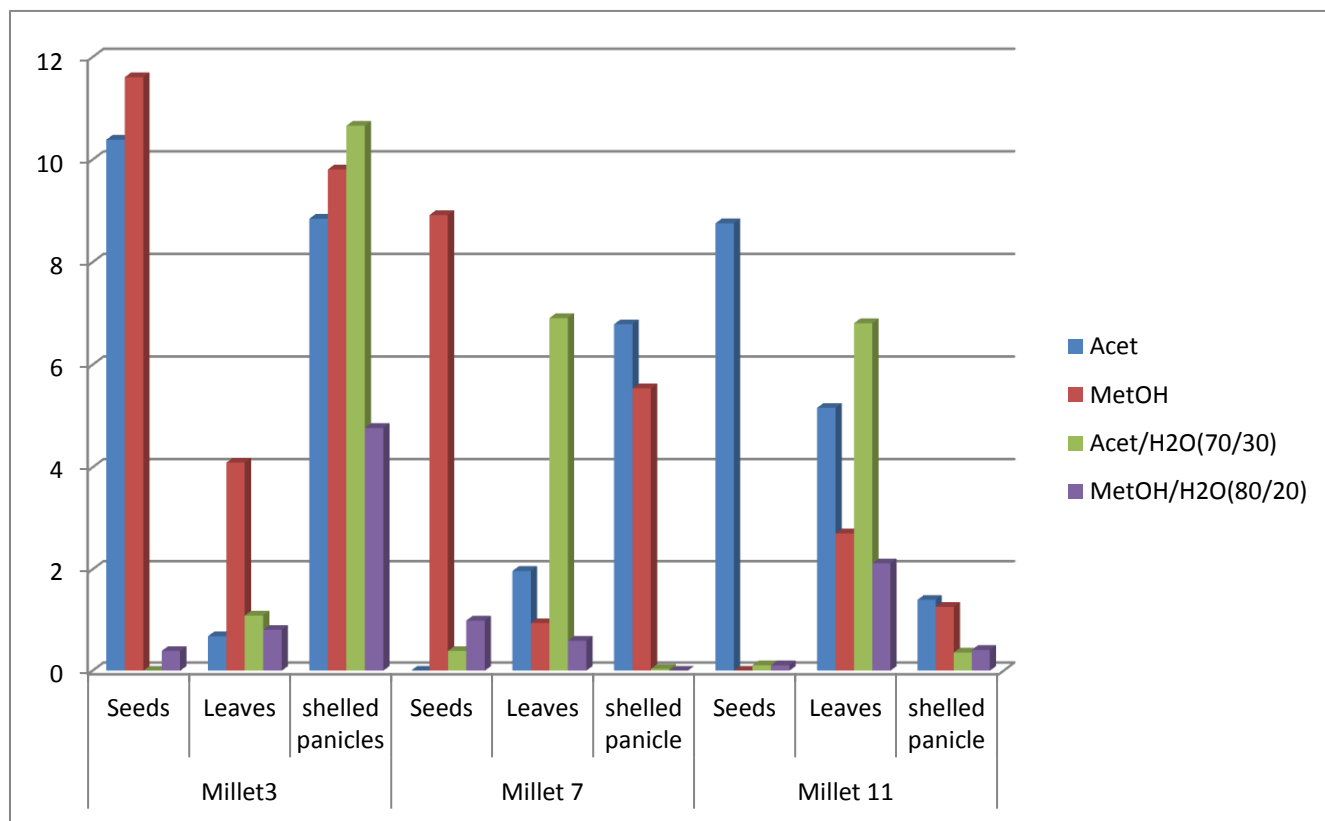


Figure 10: Comparison of scavenging activity of extracts (mg/ml) from different parts of millet on DPPH radical.

The differences in inhibition of hydroxyl radicals in different samples have shown that the extracted compositions in the extract can be responsible for hydroxyl radical inhibiting activity.

Compared to vitamin C, the extract of seeds of M3, extracted by methanol, extract of panicil shelled of M3, extracted aqueous acetone and extract of the seeds of M3 obtained by acetone have been found highest effective than these synthetic antioxidant agent .

III.3.2 (FRAP) assay

The ferric reducing ability of (FRAP) assay was used for assessing “antioxidant power” of millet extracts .The FRAP assays of extracts expressed as AEAC (ascorbic acid equivalents) are summarized in Table (07) and presented in fig(11).

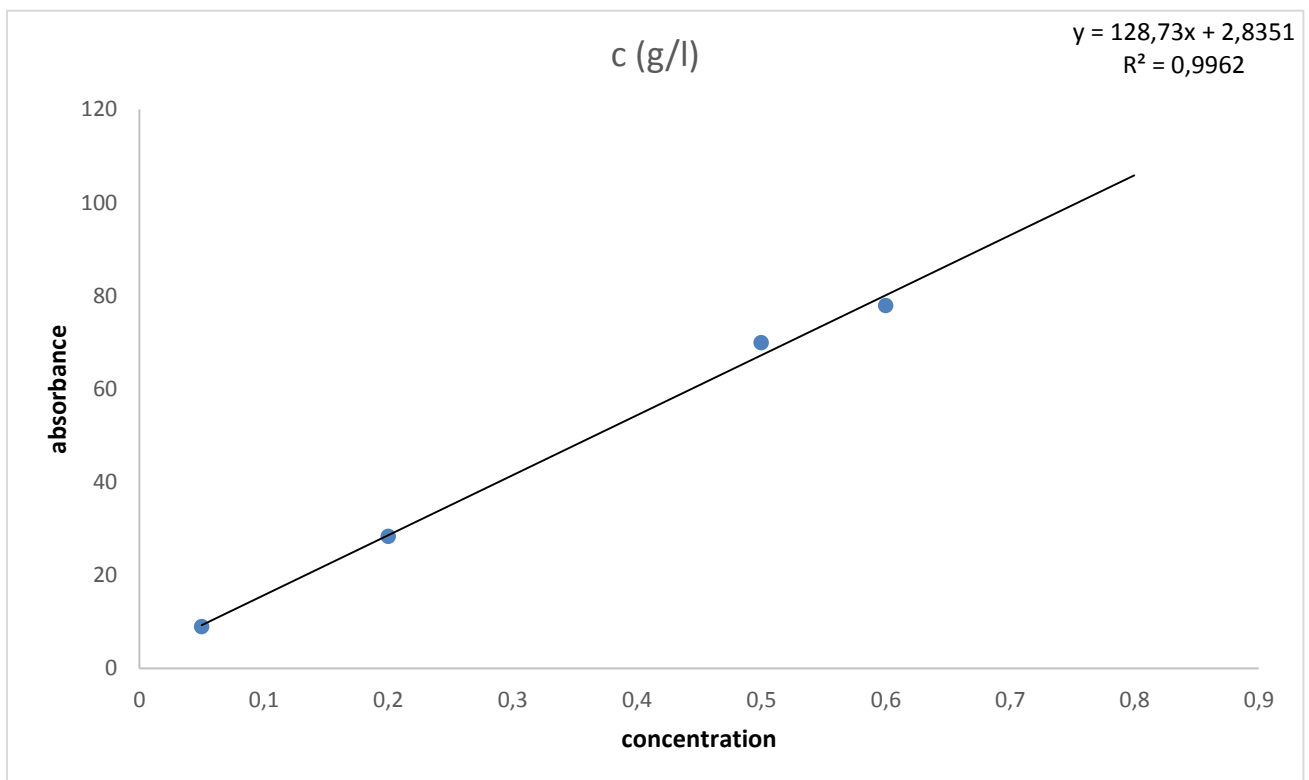


Figure 11:calibration curve ascorbic acid.

Table 7:FRAP reduction power represented by (AEAC)(g/l).

System/organ	Millet3			Millet 7			Millet 11		
	seeds	Leafs	shelled panicles	Seeds	Leafs	shelled panicles	Seeds	Leafs	shelled panicles
Acet	0.43 ± 0.01	0.26 ± 0.02	2.15 ± 1.01	0.31 ± 0.01	0.32 ± 0.03	0.4 ± 0.03	1.2 ± 0.06	0.29 ± 0.08	0.27 ± 0.10
MetOH	1.36 ± 0.03	1.66 ± 0.07	2.34 ± 1.03	0.86 ±	0.82 ± 0.03	0.65 ± 0.04	1.73 ± 0.19	0.69 ± 0.06	1.01 ± 0.02
Acet/H ₂ O(70/30)	0.35 ± 0.04	0.93 ± 0.02	2.12 ± 0.12	0.75 ± 0.03	2.08 ± 0.08	0.77 ± 0.06	0.6 ± 0.01	0.58 ± 0.03	0.82 ± 0.04
MetOH/H ₂ O(80/20)	0.64 ± 0.07	1.86 ± 0.03	1.58 ± 0.09	0.8 ± 0.01	0.75 ± 0.01	0.36 ± 0.02	1.27 ± 0.11	0.88 ± 0.09	0.76 ± 0.02

The results of the table allowed us to study the influence of solvent on the antioxidant activity. The greatest antioxidant activity was observed in methanol extract (1.36 to 2.34 mg/ml), and (0.69 to 1.73 mg/ml) from M3 and M11 respectively, followed by Acet/H₂O(70/30) (0.35 to 2.12 mg/ml) from M7 and methanol :water (0.64 to 1.86 mg/ml) from M3. The lowest AEAC was obtained by acetone extracts (0.26 mg/ml).

Among all the extracts analyzed, shelled panicles extract from M3 had the greatest antioxidant activity with a means 2.34 mg/ml, it is nearly 2 times more active than ascorbic acid. followed by seeds extract from M11 and leaves extract from M3 which are approximately 1.5 times more active than vitamin C. The shelled panicles extracts of M7 had the lowest antioxidant activity (AEAC = 0.31mg/ml).table(08) Panicles extract from M3 is approximately 4 times of shelled panicles extracts of M7 fig 10, the highest, and the lowest antioxidant activity respectively. Finally, the test of FRAP assay reveals that the extracts are Very promising source of natural antioxidants .

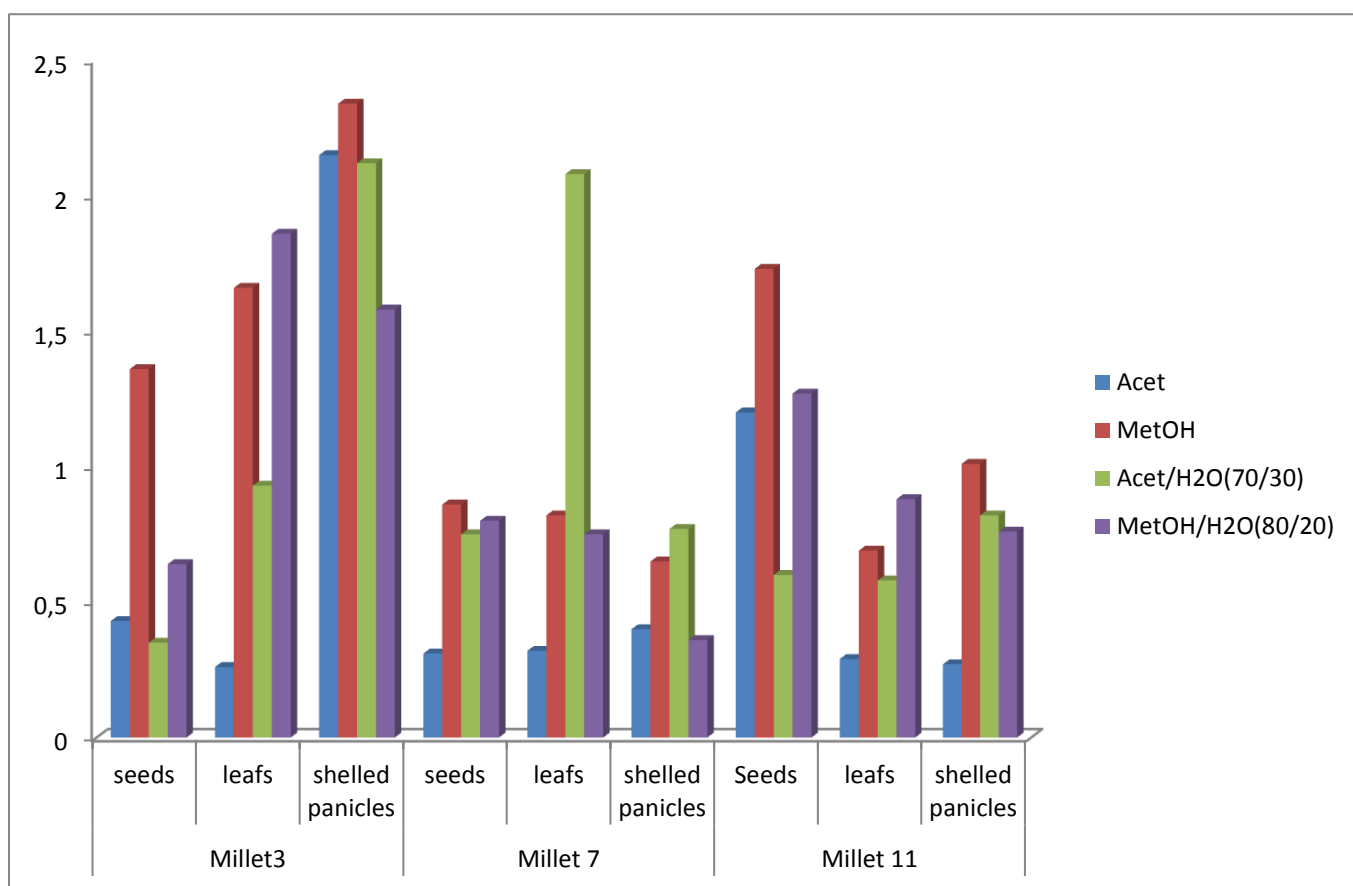


Figure 12: Comparison of frap reduction power of the extracts represented by (AEAC).

III.4 Statistical analysis

A statistical analysis was performed by using the ANOVA applying a significance level of $p \leq 0.05$. The analysis by four different extraction systems, showed that there are significant differences in all the parameters studied, except that there is no significant difference in FRAP (appendix 1).

The analysis of variance (ANOVA) of all the extracts obtained from the three parts of the plant, indicated that there were only two significant differences that are represented in TPC and DPPH a significance level of $p \leq 0.05$. While it was not significant in tannins and FRAP (appendix 1).

The analysis of variance (ANOVA) of all the extracts obtained from the three local varieties revealed highly significant differences between different parameters studied ($p \leq 0.05$) (Appendix 1).

III.4.1 influence of extraction system

If we are interested in the influence of the solvent factor (table03), the synthesis of all the results obtained during the quantification of total phenols, indicated that the levels of phenolic compounds spread between (0.11 and 1.53mgEAG/g) in the system acetone. the content of phenolic compounds in the system methanol varied from(0.69 to 2.48 mgEAG/g).

The highest content of phenolic compounds from the methanol (80/20) was the leaves of M11 (2.52 mgEAG/g), while the lowest content is noticed in the shelled panicles of millet7 (0.12 mgEAG/g).

In the acetone:water 70/30, the values of phenolic compounds varied between (0.15 and 2.72 mgEAG/g) . the highest value in phenolic compounds is found in the leaves of M11.fig07

Generally, these high results may be due of using methanol and acetone as a solvent, because are the most suitable solvent for the extraction of phenolic compounds due to its ability to inhibit the reaction of polyphenols oxidase that cause the oxidation of phenolic. (CHENGUEL Aouatef ,(2019)).

III.4.2 influence of organ from pearl millet plant

If we are interested in the influence of the organ factor, the leaves were remarkably high in phenolic content compared to the other organs whatever the solvent and the variety studied with values varied between (0.27 to 2.77 mgEAG/g) while shelled panicles possessed the lowest content with content varied from (0.11 to 1.53 mgEAG/g) . The seeds were intermediate in total phenols compared with leaves and shelled panicles. Fig07

For tannin concentration, the seeds extracted by methanol gave the best results with (11.61EC/g) followed with the shelled panicles by (acetone/water:70/30) (10.66 EC/g) then the seeds by (acetone) (10.39 EC/g).

These variations are explained by the difference in the chemical composition between the tissues of the organs of the varieties studied.

III.4.3 Influence of the variety of pearl millet

In order to select the appropriate variety, M11 and M3 are similar in total phenols content ranging from 0.11 to 2.72 mgGAE/g. in M11 and from 0.27 to 2.48 mgGAE/g in M3. M7 contained about 2.3 times less phenolics as compare to M11 and 1.5 times than M3. This suggests that the milling process might have removed a significant amount of polyphenols. (Youngmin Choi(2007))

The tannin content is highest in the samples of M3 with the values 11.62 EC/g and 10.66 EC/g respectively while the lowest content tannin in M 7 was the lowest with (0.03EC/g)

III.4.4 Comparison: (Ain saleh and Bourdj bouarreridj extracts)

We compared the obtained results according to the results of Ain Saleh millet, studied under the same conditions and in the same way, we concluded the comparison shown in the tables (08,09).

Table 8: Comparison between the phytochemical analysis and antioxidant capacities of Ain saleh and Bourdj bou arreridj.

	Ain saleh samples			Bordj bou arreridj samples		
	Minimum	Maximum	Moyenne	minimum	Maximum	Moyenne
PTmgTAE	0.00	3,45	1,1592	0.11	3.03	1.0276
taninsmgEC	0,00	11,60	3,0628	0.00	11.98	3.5079
FRAPumol	0,06	6,58	1,9785	1.14	20.31	5.4786
DPPH umol	0,03	6,00	2,5569	0.20	11.42	3.6262

the table shows converged results when it comes to (TPC) an (tannins) between Ain saleh and Bourdj bou arreridj while for the (DPPH) and (FRAP) capacities the highest results was recorded for Bourdj Bou arreidj samples.

Table 9: represents the organ effect on the (TPC) tannins FRAP and DPPH in Ain saleh and Bourdj bou arreridj samples

Region	Ain saleh samples			Bordj bou arreridj samples		
PT (mg TAE/g)	0,77±0,08 (a)	0,90±0,09 (a)	1,80±0,20 (b)	0.81 ± 0.40 (a)	0.64 ± 0.50 (a)	1.62 ± 0.89 (b)
Tannins(mg EC/g)	2,36±0,61 (a)	3,03±0,64 (a)	3,80±0,60 (a)	3.54 ± 4.68 (a)	4.16 ± 4.02 (a)	2.82 ± 2.32 (a)
FRAP (µmol/g)	1,50±0,35 (a)	1,70±0,25 (a)	2,72±0,20 (b)	4.88 ± 2.50 (a)	6.28 ± 4.87 (a)	5.26 ± 3.56 (a)
DPPH (µmol/g)	1,71±0,32 (a)	2,75±0,21 (a,b)	3,20±0,36 (b)	4.31 ± 3.34 (b)	1.67 ± 1.36 (a)	4.88 ± 2.84 (b)

a :signifiant

b:signifiant

(A,b) : significatif with a and b.

The table clarify the (TPC) tannins content in the seeds leaves and the panicle shelled also (FRAP) reduction power and (DPPH) capacity to Ain saleh and Bourdj bou arreridj samples.

This table reassure that the pearl millet contains high content of polyphenols and tannins and the leaves have the highest polyphenols content.

III.4.5 Pearson's correlation studies:

Correlation analysis is used to check the association between total phenolic content , condensed tannin content and antioxidant potential (DPPH, FRAP) of the 36 extract from 3 varieties , it gives permission to study the association or the dependence of two variables or more it's characterized with correlation coefficient that varies between -1 and +1 where the more the coefficient is close to +1 the more the linear positive relation is strong , and the more it's close to -1 the more the linear negative relation is strong . also we check the significance to know whether the results are important in our context or not where ; P-value ≥ 0.05 (insignificant) and P-value ≤ 0.05 (significant).

Table 10: Pearson's correlation coefficient between total phenolic content, condensed tannin content and antioxidant potential (DPPH, FRAP) of the 36 extract from 3 varieties.

	PT mgTAE	Tannins mgEC	FRAP mg	DPPH eqmg
PT mgTAE	1			
Taninsmg EC	0,188	1		
FRAP mg	0,371**	0,454**	1	
DPPH eqmg	0,674**	0,112	0,300*	1

** . the correlation is significative at 0.01 (bilatéral).

*.the correlation is significative at 0.05 (bilatéral).

Polyphenols contents (TPC) showed a moderate positive and significant correlation with FRAP and DPPH (0.371 and 0.677) when P-value is less than 0.01 (Table 08). As a result, the (TPC) is related to the antioxidant activity. Tannins contents also showed significant and

moderate positive correlation with FRAP (0.455) when P-value is less than 0.01. Also, we showed significant and low positive correlation with FRAP and DPPH (0.3) when the P-value is less than 0.05 (Table10). As a result the tannin content is related to the FRAP and it's not related to DPPH scavenging ability.

Conclusion

IV. Conclusion

This study aims to evaluate the extracts of panicles, grains and leaves of pearl millet varieties which belongs to the Bordj Bou Arreridj area. This evaluation is based on the extraction and quantification of phenolic compounds and tannins using four solvent systems (acetone, acetone/water, methanol, methanol/water) on the one hand, and the evaluation of the antioxidant activity of the extracts on the other hand

The results obtained show that the plant is considered an important source of phenolic compounds. The system (acetone/ water 70/30) was the best for the extraction of phenolic compounds, and the methanol system was the best for the extraction tannins. The extracts obtained with the system (methanol) were recorded as good inhibition capacity, also most of the extracts from the system (methanol) were recorded as the best reduction capacity.

The results were differentiated for the cultivars, so that millet 3 is considered the richest in phenolic content, and it also recorded the best ability to inhibit free radicals compared to the cultivars studied, followed by millet 11, which is also rich in phenolics and tannins, but has recorded good reducing capacity.

Correlation coefficient values between phenolic compounds and the ability to inhibit free radicals show that phenolic compounds contribute to antioxidant capacity, while coefficient values between tannins and reducing capacity show that tannins contribute to antioxidant capacity reductive.

Perspective

We suggest to use qualitative and quantitative analysis such as CPG and HPLC to determine and quantify the compounds in our crude extracts.

Also to purify the extract and quantify the new tannins content by changing the quantification method or changing the standard, and evaluating their antioxidant activity to compare the the results with our crude extracts.

List of references

- A. Chandrashekar, K.V. Satyanarayana,(2006).** Disease and pest resistance in grains of sorghum and millets. *Journal of Cereal Science* 44 (2006) 287–304
- Benzie, I. F. F., &Strain, J. J. (1996).** The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry*, 239, 70-76
- Blois, M.S. (1958)** Antioxidant determinations by the use of a stable free radical. *Nature* 181(4617), 1199-1200.
- Boizot.N, Charpentier, J.P. (2006).** A rapid method for the evaluation of the content of phenolic compounds of the organs of a forest tree. *Methods and tools for observation and assessment of forestiens environments grassland, and aquatic, INRA*, 79-82
- CHENGUEL Aouatef ,(2019)**Phytochemical study and biological activity of different extract from flowers of parasitic plant *Cistanche tinctoria* (Desf.) Beck. Master's thesis. El-chahid Hamma Lakhdar El-Oued University.
- Davies KJ. Oxidative stress:** the paradox of aerobic life. *Biochem Soc Symp* 1995;61:131.
- density in pearl mille. *Journal of Cereal Science* 79 (2018) 247–252.
- Kaifes,C et Bessas,N(2021).**Influence de la date de la récolte sur la teneur des composés phénoliques et l'évaluation des activités antiradicaiareset inhibitrice de α -amylase sur les extraits des feuilles de *Pistacia atantic*.Desf,université Amar Telidji Laghouat.
- Khaoula BEN HAOUA:** Quantification, caractérisation, et propriétés antioxydantes des polyphénols dans la pomme de terre de la région d'El Oued, thèse de doctorat en chimie, Université Mohamed Khider Biskra.
- Krishnaiah D, Sarbatly R, Bono A (2007)** Phytochemical antioxidants for health and medicine: A move towards nature. *Biotechnol Mol Biol Rev* 1: 97-104.
- Kunwar A, Priyadarsini KI.** Free radicals, oxidative stress, and importance of antioxidants in human health. *J Med Allied Sci* 2011;1:53-60.
- Linda Dykes, Lloyd W. Rooney,(2006).** Sorghum and millet phenols and antioxidants. *ournal of Cereal Science* 44 (2006) 236–251.
- Mahalingam Govindaraj*, Kedar N Rai, Anand Kanatti, Harshad Shivade (2018)** Terminal drought and a d2 dwarfing gene affecting grain iron and zinc
- Mahantesh S.P, Gangawane A.K, PATIL C.S,** free radicals, antioxidants, diseases and phytomedicines in human health: future prospects, *World Research Journal of Medicinal & Aromatic Plant*, 2012; (1) pp.-06-10.

measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry*, 239, 70-76

Mrabti, H.N. (2018). Etude pharmacologique Toxicologique de l'Arbutus unedo L. au Maroc. Thèse de doctorat, université Mohammed V de Rabat, Morocco. MTCC-548. *Resource-Efficient Technology* 2(2016)148-157.

NUUTILA A. M., PUUPPONEN-PIMIA R., AARNI, M., & OKSMAN CALDENTEYK. M., 2003 - Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. *Food Chemistry*, 81. P: 485-493.

Oyaizu, M. (1986). "Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine." *The Japanese journal of nutrition and dietetics* 44(6):307-315

Pitchaon Maisuthisakul a , Maitree Suttajit b , Rungnaphar

Pongsawatmanit (2007) Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants *Food Chemistry* 100 (2007) 1409–1418

Prashant S. Hegde, Namakkal S. Rajasekaran, T.S. Chandra .(2005). Effects of the antioxidant properties of millet species on oxidative stress and glycemic status in

Prashant S. Hegde, T.S. Chandra (2005). ESR spectroscopic study reveals higher free radical quenching potential in kodo millet (*Paspalum scrobiculatum*) compared to other millets. *Food Chemistry* 92 (2005) 177–182

Price Et Al, 1978: Price, M.L; Van Scoyoc, S; Butler, L.G. (1978). A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *J. Agr. Food Chem.* 26:1214-1218

PRIOR R. L., WU X. L & SCHAICH K., 2005 - Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, 53(10). P: 4290-4302

Raj Kumar Salar a,*, Sukhvinder Singh Purewal a , Manpreet Singh Bhatti (2016). Optimization of extraction conditions and enhancement of phenolic content and antioxidant activity of pearl millet fermented with *Aspergillus awamori*

Sharma, O.P. and Bhat, T.K. (2009) DPPH antioxidant assay revisited. *Food Chemistry*,

Singleton et Ross en 1965: Singleton V.L., Rossi J.R, Colorimetry of total phenolics with phosphomolybdic-phosphostungstic acid. *American journal of Enology and viticulture.* 1965, 144.

Youngmin Choi, Heon-Sang Jeong, Junsoo Lee, (2007) Antioxidant activity of methanolic extracts from some grains consumed in Korea. *Food Chemistry* 103 (2007) 130–138

Gougue Fatna.(2010). Valorisation de l'activité antioxydante des fractions: lipidiques, protéiques, et phénoliques du mil local .mémoire de magister. Amer telidji universite de Laghouat

Appendix 1

Table 11:the analysis of variance (ANOVA) with different parameters among the 36 .extract.

	Minimum	Maximum	Moyenne	Ecart type	Variance
PTmgGAE	0.10	2.85	0.9675	0.71997	0.518
PTmgTAE	0.11	3.03	1.0276	0.76356	0.583
taninsmgEC	0.00	11.98	3.5079	3.79743	14.421
FRAPumol	1.14	20.31	5.4786	3.76780	14.196
FRAPmg	0.20	3.58	0.9658	0.66381	0.441
DPPH umol	0.20	11.42	3.6262	2.97004	8.821
DPPHmg	0.04	2.01	0.6378	0.52032	0.271

Table 12:the analysis of variance (ANOVA) studying the solvent effect among the 36.

	System1 Ac	system 2 Ac /H	System3 Me	System4 Me/H
PTmgGAE	0.4894 ± 0.42153a	1.1200 ± 0.80752b	1.3033 ± 0.67286b	0.9572 ± 0.70421a ,b
PTmgTAE	0.5206 ± 0.44725a	1.1906 ± 0.85661b	1.3850 ± 0.71236b	1.0144 ± 0.74696a ,b
taninsmgEC	4.0111 ± 3.80210a,b	2.9283 ± 3.95969a	5.9628 ± 3.86077b	1.1294 ± 1.48041a
FRAPumol	3.5567 ± 3.57889a	5.6139 ± 4.60667a,b	7.0628 ± 3.26343b	5.6139 ± 2.82483a, b
FRAPmg	0.6272 ± 0.63090a	1.0011 ± 0.81188a,b	1.2450 ± 0.57434b	0.9900 ± 0.49745a, b
DPPH umol	2.4867 ± 3.54214a	4.5050 ± 2.61686a	4.1550 ± 3.18941a	3.3583 ± 2.17009a
DPPHmg	0.43780 ± 62274a	0.7889 ± 0.45151a	0.7317 ± 0.56122a	0.5928 ± 0.38217a

Table 13:the analysis of variance (ANOVA) studying the organ effect among the 36.

	G (seeds)	D (stems)	F (leafs)
PTmgGAE	0.7675 ± 0.38065a	0.6029 ± 0.47902a	1.5321 ± 0.84409b
PTmgTAE	0.8150 ± 0.40379a	0.6421 ± 0.50862a	1.6258 ± 0.89573b
taninsmgEC	3.5404 ± 4.68220a	4.1621 ± 4.02818a	2.8212 ± 2.32949a
FRAPumol	4.8813 ± 2.50811a	6.2858 ± 4.87182a	5.2688 ± 3.56371a
FRAPmg	0.8604 ± 0.44226a	0.9287 ± 0.85829a	1.1083 ± 0.62753a
DPPH umol	4.3163 ± 3.34014b	1.6746 ± 1.36411a	4.8879 ± 2.84265b
DPPHmg	0.8567 ± 0.58716b	0.2954 ± 0.24034a	0.758 ± 0.49497b

Table 14:the analysis of variance (ANOVA) studying the variety effect among the 36 extracts.

	M11	M7	M3
PTmgGAE	1.1696 ± 0.89576b	0.6817 ± 0.49199a	1.0512 ± 0.64828a;b
PTmgTAE	1.2525 ± 0.95026b	0.7250 ± 0.52151a	1.1154 ± 0.68776a;b
taninsmgEC	2.5046 ± 2.82680a	2.7596 ± 3.23904a;b	5.2596 ± 4.60399b
FRAPumol	4.8117 ± 2.41750a	4.1996 ± 2.65090a	7.4246 ± 4.98290b
FRAPmg	0.8488 ± 0.42556a	0.7408 ± 0.46695a	1.3079 ± 0.87857b
DPPH umol	5.3313 ± 3.81462b	2.4646 ± 2.25497a	3.0829 ± 1.68659a
DPPHmg	0.9337 ± 0.66773b	0.4346 ± 0.39814a	0.5450 ± 0.29646a

Table 15:pearson correlation.

	PTmgTAE	taninsmgEC	FRAPumol	FRAPmg	DPPHequmol	DPPHeqmg
PTmgTAE	1					
taninsmgEC	0,188	1				
FRAPumol	0,371**	0,455**	1			
FRAPmg	0,371**	0,454**	1,000**	1		
DPPHequmol	,677**	,113	,295*	,295*	1	
DPPHeqmg	,674**	,112	,300*	,300*	1,000**	1

Table 16:significance of the correlation.

	PTmgTAE	taninsmgEC	FRAPumol	FRAPmg	DPPHequmol	DPPHeqmg
PTmgTAE						
taninsmgEC	0,114					
FRAPumol	0,001	0,000				
FRAPmg	0,001	0,000	0,000			
DPPHequmol	0,000	0,344	0,012	0,012		
DPPHeqmg	0,000	0,350	0,011	0,010	0,000	

ملخص

يعتبر الدخن الولؤي نباتا غذائيا ذو فوائد عديدة , و مصدرا لانواع مختلفة من مضادات الاكسدة مثل (الفينولات , التانينات ... الخ) و التي تعمل على تثبيط الجذور الحرة داخل جسم الانسان , هذا العمل تم تكريسه لاستخراج المركبات الفينولية من اجزاء نبات الدخن الولؤس المحلي لبرج بو عريريج , تم تقسيم هذه الاجزاء الى (بذور, عناقيد و اوراق) النشاط المضد للاكسدة تم تقييمه باستعمال اختبارين كيميائيين dpph و frap . اظهرت النتائج بأن هذه الانواع المحلية تعتبر مصدرا و اعدا بالمركبات الفينولية , الاوراق المستخرجة من 11 M باستعمال الميثانول أعطت أفضل عائد ب(9.26%). كمية الفينولات المستخرجة باستعمال الاسيتون تراوحت ما بين (0.11 الي 1.53 mgGAE/g) وباستعمال الميثانول تراوحت ما بين (0.69 الي 2.48 mgGAE/g) , أما نتائج الاسيتون/ماء (30/70) و الميثانول/ماء (20/80) كانت (0.62 الي 0.72 mgGAE/g) و (0.12 الي 2.52 mgGAE/g) على التوالي , بينما كمية التانينات سجلت نتائج أعلى بالنسبة للمستخلصات الأسيوتونية و الميثانولية حيث سجلت (10.39 mgEC/g) (11.61 mgEC/g) بالترتيب , أما من أجل مستخلصات (الأسيوتون 30/70) و (الميثانول 20/80) النتائج كانت (10.66 mgEC/g) (4.77 mgEC/g) . نتائج النشاط المضاد للأكسدة كان عاليا بالنسبة لكل من dpph و frap , أوراق M3 سجلت أفضل قدرة على تثبيط الجذور الحرة (11.61 VEAC) و (AEAC2.34) لعناقيد M3 كأفضل قدرة ارجاعية frap . نتائج تحليل التباين (ANOVA) بالنسبة للمحاليل المختلفة و الأنواع المختلفة أظهرت تباينا ملحوظا مما يعني اختلافا كبيرا في نتائج العوامل المدروسة , بينما اجزاء النبات أظهرت اختلافا في العاملين (TPC) و dpph . دراسة الترابط تبرهن أن (TPC) تساهم في النشاط المضاد للأكسدة حيث ترتبط كمية التانات بال frap ولا ترتبط بالقدرة على تثبيط الجذور الحرة dpph .

الكلمات المفتاحية : الدخن الولؤي التانينات , نشاط مضاد للاكسدة , قدرة تثبيط الجذور الحرة dpph , القدرة الارجاعية frap

Résumé

Le mile perlé et un plant avec plusieurs avantages, il est considéré d'être une source des antioxydants variés (phénol totaux, les tannin ..etc) , qui travaille à inhiber les radicaux libre dans le corps humain , ce travail a été consacré à extraire les composés phénolique de différents parties de mile perlé locaux de bourdj bou arreridj , ces parties ont été divisées en (grain , déchet , feuille) l'activité antioxydante a été évaluée par deux tests chimiques FRAP et DPPH.

Les résultats montrent que ces variétés locales sont des sources prometteuses de composés phénoliques , le meilleur rendement a été trouvé dans les feuilles de M11 extraites par le méthanol avec (9.26%) les phénols totaux extraits par l'acétone varient de (0.11 à 1.53 mgGAE/g) et les extraits par méthanol varient de (0.69 à 2.48 mgGAE/g) , les résultats par (acétone / H₂O:70/30) et (méthanol:80/20) ont été (0.62 à 2.72 mgGAE/g) et (0.12 à 2.52 mgGAE/g) (0.13 à 2.68 mgTAE/g) respectivement , , alors que pour les tannins les plus hauts résultats ont été marqués par les extraits avec l'acétone et le méthanol (10.39 mgEC/g) (11.61 mgEC/g) respectivement et pour (acétone / H₂O:70/30) et (méthanol:80/20) les résultats sont (10.66 mgEC/g) (4.77 mgEC/g) .

Les résultats d'activité antioxydante ont été élevés pour les deux DPPH et FRAP, les meilleurs résultats ont été marqués par les feuilles de M3 avec (11,61 VEAC) et (2.34 AEAC) par le dichet de M3 pour le résultat de FRAP .

Les résultats d'analyse de variance (ANOVA) des solvants système et variétés montrent une variance significative ce qui veut dire qu'il y a une différence dans les paramètres étudiés alors que les différentes parties du plant montrent une variance significative dans (TPC) et DPPH.

L'étude de corrélation montre que le (TPC) contribue à l'activité antioxydante, où les tannins sont liés avec le FRAP , et pas liés avec la capacité d'inhibition DPPH.

Mots clés: mil perlé, contenu phénolique, activité antioxydante ,DPPH , FRAP.

Abstract

Pearl millet is a nutritional plant with many benefits, it's considered to be a source of various antioxidants (total phenols, tannins..etc) , that works to inhibit the free radicals effect in the human body , this work was devoted to extract the phenolic compounds from different parts of local pearl millet of bordj bou arrereidj ,this parts was divided to (seeds, panicle shelled and leafs) , the antioxidant activity was evaluated by two chemical tests FRAP and DPPH .

The results showed that these local varieties are promising source of phenolic compounds, the best yield was found in the leaves of M11 extracted by methanol with (9.26%).

The total phenolic content extracted by acetone ranged from (0.11to1.53 mgGAE/g) and by methanol ranged from (0.69to 2.48 mgGAE/g) the results by (acetone / H₂O:70/30)and (methanol:80/20) was (0.62 to 2.72 mgGAE/g) and (0.12 to 2.52 mgGAE/g) respectevly , while for tannins content higher results was recorded by acetone and methanol extracts where they mark (10.39 mgEC/g) (11.61mgEC/g) respectively and for (acetone / H₂O:70/30) and (methanol:80/20) the results was (10.66 mgEC/g) (4.77 mgEC/g) .

The results of antioxidant activity was high in both DPPH and FRAP , the best scavenging result was given by the leaves of M3with (11,61VEAC) and (2.34 AEAC) by panicle shelled of M3 for FRAP reducing result.

the variance analysis (ANOVA) results of the solvent systems and varieties showed a significant variance which means high difference in the parameters studied while the plant parts showed two significant difference in (TPC) and DPPH.

The correlation study proves that (TPC) contribute in the antioxidant activity, where, tannins content is related to the FRAP and it's not related to DPPH scavenging activity.

Key words: pearl millet, Phenolic content, antioxidant activity ,DPPH , FRAP.