



Antidiabetic, antioxidant, and phytochemical profile of *Pennisetum glaucum* cultivated in central-southern Morocco and imported from India

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ABSTRACT

Alternative methods of producing food sustainably are being considered due to rapid expanding population, increasing food needs, declining agricultural soils fertility, and ongoing drought. To achieve that, we examined the pharmacological, functional, and nutritional characteristics of two cultivars of pearl millet (*Pennisetum glaucum*), which are distinguished by their ability to tolerate a variety of climates compared to other cereal grains. In this study, we evaluated the antioxidant and antidiabetic effects of two seeds cultivars: one cultivated in Morocco and the other grown in India. In addition to having a higher energy value (403.91 Kcal), higher protein (9.13 %) and lipid content (7.11 %), and a lower capacity for self-oxidation than the Indian variety due to its fatty acid composition, the content of phenolic compounds of Moroccan millet is well concentrated in its seeds, making it more powerful as an antioxidant and hypoglycemic plant. The ethanolic extract of Moroccan pearl millet showed the greatest total phenolic content (36.278 mg GAE/g extract), DPPH and ABTS radical scavenging activity ($IC_{50} = 152.286$ and $252.480 \mu\text{g/mL}$ respectively), α -amylase and α -glucosidase inhibitory activity ($IC_{50} = 84.439 \mu\text{g/mL}$ and $224.103 \mu\text{g/mL}$ respectively). The evidence gathered in this study indicates that *P. glaucum* possesses characteristics that qualify it as a food, medicinal, and productive plant.

1. Introduction

Traditional medicine in Morocco has historically utilized various plants for maintaining well-being and preventing diseases [1]. These plants, rich in secondary metabolites like alkaloids, terpenoids, and phenylpropanoids, are used in the production of pharmaceutical drugs [2]. Morocco's aromatic and medicinal plant sector, with over 4200 floristic species, is the richest in North Africa [3]. As these plants gain popularity, functional foods have emerged, combining their nutritional and medicinal aspects. These foods contain essential nutrients that enhance human health and meet nutritional requirements [4]. Cereals, a staple food for the Moroccan population, account for 12 % of

agricultural production and consume over five million tons annually. However, drought has reduced cereal production by 25–85 %, leading to significant imports from other countries [5], such as India's imported variety of *P. glaucum*.

Pennisetum glaucum (L.) R. BR. is a monoic annual herb with extensive roots that resist drought, salinity, and high temperatures [6–8]. It is the sixth largest cereal crop in Asia and Africa, with India being the largest producer [9–11]. Pearl millet is the most widely grown variety, with high nutritional value and significant socio-economic and environmental impacts. The United Nations Food and Agriculture Organization declared 2023 International Millet Year to recognize its international significance [12]. So, pearl millet's phytochemical

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richness in active substances such as polyphenols, flavonoids, alkaloids, saponins, tannins, and anthraquinones provides a range of pharmacological properties, including antioxidant [13–17], antimicrobial [16, 18], antidiabetic [19–22], antihypertensive [23], anti-inflammatory [24], and anticholesterolemic [22] properties, protecting humans from metabolic syndrome.

In the metabolic syndrome (MS), metabolic processes in the body are dysregulated, which is characterized by a number of risk factors such as impaired insulin sensitivity, atherogenic dyslipidemia, obesity and hypertension [25–28]. Thus, MS increases the risk of type 2 diabetes by a factor of 2, and of cardiovascular disease by a factor of 3, as well as other diseases including cancer, lipid disorders and atherosclerosis, which makes it one of the world's leading causes of death [29,30]. MS is a key pathophysiology characterized by abnormal oxidative stress, redox imbalance, systemic inflammation, endothelial dysfunction, altered metabolic and DNA systems, and regenerated diseases [31]. In response to oxidative stress, significant changes occur within the cell, leading to the production of reactive oxygen species (ROS) in large quantities. These species (ROS) can oxidize cellular proteins, nucleic acids, and lipids, affecting food nutritional quality and leading to insulin resistance and hyperglycemia. However, bioactive compounds like polyphenols, flavonoids, antioxidant and antidiabetic proteins can prevent lipid peroxidation and diabetes, allowing for the creation of special diets for patients with both conditions [32–34].

Notwithstanding the variety of *P. glaucum* seeds available, only the Indian import variety is propagated in Morocco's commercial establishments. We therefore went in search of the Moroccan cultivar and compared it with the imported variety. By contrasting their phytochemical profiles and evaluating their antioxidant and anti-diabetic properties, the work's real goal was primarily concerned with identifying the nutritional, pharmacological, and qualitative parameters of Moroccan seeds and compare with those grown in India.

2. Materials and methods

2.1. Chemicals and reagents

All reagents used in the present study including the Folin-Ciocalteu reagent, Sodium carbonate (Na_2CO_3), gallic acid, sodium nitrite (NaNO_2), aluminum trichloride (AlCl_3), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), and iodine solution were of analytical grade. Tocopherol isomer standards (with $\geq 98\%$), p-nitro-phenyl- α -D-glucopyranoside (p-NPG), α -amylase and α -glucosidase were supplied by Sigma-Aldrich Co. (St. Louis, MO, USA). Solvents such as ethanol, hexane, isooctane, isopropanol, and cyclohexane were of HPLC quality. Other solvents used were of analytical grade.

2.2. Botanical matter

Two cultivars of *P. glaucum* were used in the current research; one cultivated in Morocco in the Marrakech region and characterized by small, yellow-brown seeds, and one imported from India (IPM). This cultivar is highly regarded in Moroccan markets and is distinguished from the other cultivars by its grayish color. A thorough cleaning and sorting of the samples was carried out, as well as the removal of foreign particles.

2.3. Proximate analysis

2.3.1. Crude fat

The oil was extracted from 20 g of powdered seeds using a Soxhlet system for 6 h with pure *n*-hexane (200 mL). Solvent was evaporated in a vacuum rotary evaporator operating at low pressure. Up to a subsequent examination and gravimetric calculation of the extraction yield (oil), the oil was kept at $-4\text{ }^\circ\text{C}$ [35].

2.3.2. Moisture content

To determine the specific water content of each individual sample, the weighed samples were dried in an oven at $103\text{ }^\circ\text{C}$ until they attained a stable weight. All tests were carried out in triplicate in conformity with ISO 662: 2016 [36], and means are reported.

2.3.3. Ash content

In order to determine the ash content of the seed under test, we conducted the test in accordance with ISO 18122: 2022 [37]. A crucible was filled with 5 g of each sample, and the melting pots are then transferred to a muffle oven regulated at $500\text{ }^\circ\text{C}$ for 4 h to obtain off-white ash. The pots are subsequently weighed once they have cooled in a desiccator.

2.3.4. Protein content

Crude protein was quantified using the micro-Kjeldahl procedure based on total nitrogen [38].

2.3.5. Total carbohydrates content

As specified by Gagour et al. [35], the calculation of carbohydrates was made by removing crude fat, moisture, ash, and protein content from 100 %.

$$\text{Carbohydrate content}(\%) = 100 - (\text{Ash} + \text{Moisture} + \text{Protein} + \text{oil content}) \quad (1)$$

2.3.6. Energy value

We calculated the energy values of the products based on the amount of protein, carbohydrate, and fat present in the product, using factors of 4, 4, and 9 kcal/g, respectively [16].

$$\text{Energy value (Kcal)} = 4 * \text{Proteins} + 4 * \text{carbohydrate} + 9 * \text{lipids} \quad (2)$$

2.3.7. Water and oil absorption capacity

In accordance with John et al. [39]. One gram of each flour (MPM and IPM) was mixed vigorously with 10 mL of purified water or purified sunflower oil and left to stand at ambient conditions for 30 min. In a weighted centrifuge tube, the resulting blend underwent a 10-min centrifugation at 2000g, and the supernatant was decanted. The mass of the sample just prior to settling was compared to the following settling in order to compute WAC and OAC.

2.3.8. Bulk density

Each flour sample was placed in a graduated cylinder of one hundred milliliters (100 mL) containing fifty (50) g of the corresponding sample. On a laboratory bench, the test tube was struck several times at a constant volume level before being recorded. After tapping, the sample weight per sample volume was used to determine the bulk density (g/cm^3) [40].

2.3.9. Swelling capacity

The samples were placed in graduated cylinders of one hundred mL, each filled to the 10 mL mark. Distilled water was then added to bring the amount down to 50 mL. By flipping the cylinder, the graduated cylinder tops were snugly covered and combined. After 2 min, the suspension was inverted once more, and it was left to stand for an additional 30 min. After 30 min, the sample's volume was measured [41].

2.4. Chemical composition and properties of lipidic fraction

2.4.1. Chemical composition

The American Oil Chemists' Society (AOCS) method [42] was used to determine saturated, monounsaturated and polyunsaturated fatty acids. After being transesterified with KOH/methanol, the fatty acids (FAs) were transformed into FAMES, which were then subjected to analysis by gas chromatography using a capillary column (Varian CP-3800). A polar

stationary phase and split inlet configuration were used, and the separation was determined by the length of the fatty acid chain, degree of unsaturation, or position of double bonds (also known as position isomers). Approximately 30 m wide and 32 cm high were the dimensions of the fused silica columns. Helium was employed as the carrier gas in chromatography, and temperatures reached 170 °C at the start of the column and 230 °C by the end; the rate of increase was 3 °C/min. Using the FID detector at room temperature, they detected a 1L injection and expressed the percentages of each individual fatty acid in their sample. The results were presented as separate tables for comparison.

In addition to fatty acids, sterol levels and composites were assessed by GC, in accordance with the guidelines of AOCS official method Ch 6–91 [43]. To summarize, a fifty (50) mg lipid sample underwent saponification with methanolic KOH (1 M) at ambient conditions for eighteen (18) h. Water was subsequently added and the unsaponifiable components were extracted three times with a mixture of hexane and *tert*-butyl methyl ether (1:1, v/v). We separated silylated by Sylon BTZ derivatives of sterols using a Hewlett Packard 6890 gas chromatograph equipped with a DB-35MS capillary column distinguished by a length of 25 m, an internal diameter of 0.20 mm and a film thickness of 0.33 µm. The test sample was split-injected. An internal standard, 5 α -cholestane, was employed for the quantification of sterols. Phytosterols were distinguished by comparing their retention data to that of the standard.

Lastly, the tocopherols amount and compositions were calculated using HPLC, as per the protocol described in AOCS Official Method Ce 8–89 [44].

2.4.2. Physicochemical properties

Free fatty acid (FFA), peroxide value (PV), and UV specific extinction coefficients (K232 and K270) are examples of the physicochemical quality parameters that were determined using the authoritative methodological approaches ISO 660:2020 [45], ISO 3960:2017 [46], and ISO 3656:2017 [47].

We evaluated the pigment content of the extracted oil samples using the method outlined in Gagour et al. (2022) [35]. Each sample was precisely weighed to 7.5 g and then dissolved in cyclohexane to reach a final volume of 25 mL. The absorbance at 470 and 670 nm was used to measure the pigments carotene and chlorophyll. In the following equations, A represents absorbance.

$$\text{Chlorophyll} \left(\frac{\text{mg}}{100\text{g}} \right) = \left(\frac{A_{670} \times 10^6}{613} \right) \times 100 \quad (3)$$

$$\text{Carotenoid} \left(\frac{\text{mg}}{100\text{g}} \right) = \left(\frac{A_{470} \times 10^6}{2000} \right) \times 100 \quad (4)$$

$$IT = \frac{(C18:0 + C14:0 + C16:0)}{[(0.5 \times MUFA) + (0.5 \times n6PUFA) + (3 \times n3PUFA) + (\sum n3PUFA / \sum n6PUFA)]} \quad (10)$$

2.4.3. Qualitative, nutritional and metabolic indexes

In most cases, saturated and unsaturated fatty acid are derived from a combination of nutritional supplements, each of which has a unique fatty acid composition and therefore affects a variety of health conditions. For this reason, it is necessary to examine fatty acid profiles accurately in order to classify foods according to their functional and nutritional qualities, particularly in the case of fatty acid-rich foods, dietary supplements and herbal remedies.

The most popular metric for assessing how a specific diet affects cardiovascular health is the Polyunsaturated Fatty Acid/Saturated Fatty Acid (PUFAs/SFAs) index. This index is based on the assumption that while all SFAs have the potential to raise serum cholesterol, all PUFAs can lower serum cholesterol and low-density lipoprotein cholesterol

(LDL-cholesterol) [48,49].

$$\frac{PUFAs}{SFAs} = \frac{\sum \text{Polyunsaturated Fatty Acids}}{\sum \text{Saturated Fatty Acids}} \quad (5)$$

The Linoleic Acid/ α -Linolenic Acid (LA/ α -LA) Ratio was created as a means of assessing the nutritional value of baby formula. Because the mammalian body is unable to synthesis these two fatty acids, consuming food is the only way to receive them. Additionally, they fight for the same elongase and desaturase enzymes, which are necessary for the synthesis of LC-PUFAs [49,50].

$$\frac{LA}{\alpha LA} = \frac{C18:2n6}{C18:3n3} \quad (6)$$

The unsaturation index (UI) supplies an indication of the level of desaturation of fatty acids, rather than their simple total. In fact, the unsaturation index is an indication of the degree of unsaturation of the FA and is expressed as the total of each UFA (%) multiplying by the number of its double bonds, thereby assigning various values to the various unsaturation categories [49,51].

$$\text{Unsaturation Index} = (\% \text{monoenoic}) + (2 \times \% \text{dienoic}) + (3 \times \% \text{trienoic}) + (4 \times \% \text{tetraenoic}) + (5 \times \% \text{pentaenoic}) + (6 \times \% \text{hexaenoic}) \quad (7)$$

The Nutritional Value Index (NVI) was developed by Chen and colleagues [48] and takes into account only the most important FAs in a food of animal origin: oleic, palmitic, and stearic acids.

$$NVI = \frac{C18:0 + C18:2n9}{C16:0} \quad (8)$$

Index of atherogenicity (IA), indicates the ratio between the sum of SFAs, except stearic acid (C18:0), which the authors consider not proatherogenic because of the human ability to desaturate it to oleic acid (C18:1n-9). In contrast, palmitic, myristic and lauric acids promote lipid adhesion to vascular and immune cells and atherogenic plaque accumulation, and reduce phospholipid and esterified FA levels [48,52,53].

$$IA = \frac{[C16:0 + (4 \times C14:0) + C12:0]}{\sum UFA} \quad (9)$$

Index (IT), characterizing the thrombogenic potential of FAs, dividing them into prothrombotic (C14:0, C16:0, and C12:0) and antithrombotic FAs, such as MUFA, n-3 and n-6 PUFA, although recent studies have demonstrated the negative impact of n-6 PUFA on thrombosis [49,52,54].

The hypocholesterolemic/hypercholesterolemic (hypo/Hyper) ratio indicates the relationship from dietary FAs into low-density plasma lipoproteins in their ratio of C18:1n-9 and PUFAs (hypocholesterolemic FAs) to sum of C16:0, C12:0 and C14:0 (hypercholesterolemic FAs) [49,55].

$$\frac{\text{hypo}}{\text{Hyper}} = \frac{C18:1 + \sum PUFA}{C12:0 + C14:0 + C16:0} \quad (11)$$

The Health Promoting Index assesses the nutritional value of fat, focusing on the effects of FAs on cardiovascular disease [49,56].

$$HPI = \frac{\sum UFA}{C12:0 + (4 \times C14:0) + C16:0} \quad (12)$$

The ratio of C18:0 to C16:0 has been computed as the elongase index [49].

$$\text{Elongase} = \frac{\text{C18 : 0}}{\text{C16 : 0}} \times 100 \quad (13)$$

In many studies, calculated desaturase activities are used as estimators of true desaturase activity, and Vessby and co-authors [57] have shown that their calculated activities can be used as surrogates for measuring true desaturase activity.

$$\delta 9 - \text{desaturase (C18 : 1)} = \frac{\text{C18 : 1n9}}{\text{C18 : 0} + \text{C18 : 1n9}} \times 100 \quad (14)$$

Due to the absence of results for the qualitative, nutritive, and metabolic indices, we resorted to calculating them based on the fatty acid profile of millet and oat, for the purpose of comparing with our results.

2.5. Polyphenols and flavonoids content

Using the identical process as the hexane extraction, the powder was further extracted with ethanol to remove any remaining residue. By utilizing this fraction, we can determine its phenolic compound levels and evaluate its biological properties.

In accordance with Ait Bouzid et al. [58], the determination of TPC was performed using the Folin-Ciocalteu reagent. A 0.5 mL extract solution was first mixed with 2.5 mL Folin-Ciocalteu (1/10), then sodium carbonate Na_2CO_3 (7.5 %) was added. Following a 30 min incubation period at 45 °C, the solution was measured at a wavelength of 765 nm. Based on the calibration curve using gallic acid (GA) as a standard, results were given in mg GA equivalent per g of dry material (mg GAE/g dry material).

Using aluminum trichloride as a method, total flavonoid content was measured as outlined by EL Ouafy et al. [59]. The ethanolic extract in 1 mL first diluted with 6.4 mL distilled water was then diluted with 0.3 mL NaNO_2 solution (5 %) to form the solution. After 5 min, 0.3 mL aluminum trichloride solution (10 %) was mixed in, followed by 2 mL NaOH (1 M) after a further 5 min. A blank solution was used to measure absorbance at 510 nm, and results were given in mg quercetin equivalent (QE) per gram of crude extract.

2.6. Antioxidant activity

Based on measuring the extracts' capacity to scavenge free radicals, the antioxidant activity was ascertained using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, albeit with some adjustments, as reported by El Ouafy et al. [60]. Using an amber bottle to shield it from sunlight, a DPPH ethanolic solution (0.2 mM) was made. Using ethanol (1 mg/mL), seed polar extracts were diluted. To 0.5 mL of DPPH solution, 2.5 mL of diluted sample compound were fed. A half-hour was then spent incubating the mixture in the dark. With the aid of a UV spectrophotometer, the absorbance was determined at 517 nm in relation to a blank. The following formula was used to calculate the percentage of free radical scavenging activity (%RSA) by DPPH:

$$\% \text{RSA} = \frac{\text{Acn} - \text{At}}{\text{Acn}} \times 100 \quad (15)$$

Where RSA denotes the radical scavenging activity, At denotes the absorbance of the mixture solution containing the extract and DPPH and Acn denotes the absorbance of the mixture solution containing ethanol and DPPH.

According to EL Mouddeh et al.'s technique [61], the ABTS radical was ascertained. For 12–16 h, equal amounts (100 μL) of the ABTS (2 mM) and potassium persulfate (70 mM) solution were kept at room temperature in the dark. 200 μL of this solution (2 mL) were added to extracts at various concentrations. After 30 min, a UV spectrophotometer was used to measure the absorbance at 734 nm.

Table 1

Proximate and functional properties of two cultivars *Pennisetum glaucum*^{a, b}.

	MPM	IPM
Crude fat (%)	7.11 ± 0.19 ^a	5.11 ± 0.237 ^b
Moisture (%)	6.25 ± 0.25 ^a	6 ± 0.05 ^a
Dry matter (%)	93.75 ± 0.25 ^a	94 ± 0.05 ^a
Ash (%)	1.66 ± 0.139 ^a	0.98 ± 0.044 ^a
Protein content (%)	9.13 ± 0.240 ^a	4.35 ± 0.516 ^b
Carbohydrate content (%)	75.85 ± 0.416 ^a	83.56 ± 0.609 ^b
Energy value (Kcal)	403.91 ± 0.626 ^a	397.63 ± 1.547 ^b
WAC (g/g)	1.61 ± 0.242 ^a	1.41 ± 0.151 ^a
OAC (g/g)	1.06 ± 0.04 ^a	0.865 ± 0.085 ^a
Swelling capacity (%)	13.75 ± 0.25 ^a	12.75 ± 0.25 ^a
Bulk density (g/cm ³)	0.769 ± 0.00 ^a	0.781 ± 0.00 ^a

^a The values of compounds are expressed as mean ± standard deviation. Values within each row with different superscript letters (a-b) are significantly different at $p \leq 0.05$.

^b Abbreviations are: MPM, Moroccan pearl millet; IPM, Indian pearl millet; WAC, Water absorption capacity; OAC, Oil absorption capacity.

2.7. Antidiabetic activity

2.7.1. α -amylase inhibition test

With minor adjustments, the starch-iodine technique from El Hachlafi et al. [62] was used to investigate the inhibition of α -amylase. To generate a range concentration of 50–500 $\mu\text{g}/\text{mL}$, a concentration of 1000 $\mu\text{g}/\text{mL}$ of each extract was made in ethanol and diluted in phosphate buffer (pH 6.9). In summary, 100 μL of phosphate buffer solution (20 mM, pH 6.9) containing the enzyme α -amylase (3U/mL) was combined with 250 μL of each sample. After 10 min of 37 °C incubation, 600 μL of 1 % substrate was added, and the mixture was incubated for a further 30 min at 37 °C. We used 600 μL of 0.1 M HCl to halt the enzymatic reaction after adding the substrate. Following the addition of 500 μL of a 25 mM iodine solution, the amount of total and residual starch was measured at 630 nm. As a positive control, acarbose was employed. The formula used to determine the percentage of inhibition was as follows:

$$\text{Inhibitory activity (\%)} = \frac{(A - B) - (C - D)}{(A - B)} \times 100 \quad (16)$$

Where, A is the absorbance of incubated solution containing substrate; B is the absorbance of incubated solution containing substrate and enzyme; C is the absorbance of incubated solution containing sample and substrate; D is the absorbance of incubated solution containing substrate, sample, and enzyme.

2.7.2. α -glucosidase inhibition test

P. glaucum cultivars were tested for their alpha-glucosidase inhibitory effects using the method described by Benrahou et al. [63] with some modifications. α -Glucosidase from *Saccharomyces cerevisiae* was used as the enzyme. P-nitrophenyl- α -D-glucopyranoside (ρ NPG) was hydrolyzed by α -glucosidase to release the ρ -nitrophenyl. A mixture of 150 μL of extract solution and 100 μL of enzymatic solution was prepared (α -glucosidase). An incubation period of 10 min was followed by the addition of 200 μL of ρ NPG as the substrate to the reaction mixture. Afterwards, the reaction was incubated for 30 min, and at the end, 1 mL of 0.1 M Na_2CO_3 was added to stop the reaction. An optical spectrophotometer at 405 nm was used to measure the concentration.

2.8. Statistical analysis

Analysis of data was done with GraphPad Prism 10. The tests were run three times, and the mean of the triplicates ± SD was used to represent the findings. Sidak's test was conducted after the means of the groups were compared using Two-way analysis of variance (ANOVA). When $p < 0.05$, differences were deemed significant.

Table 2Fatty acid, phytosterol and tocopherol composition of two cultivars of *Pennisetum glaucum*^{c, d}.

	MPM	IPM
Fatty acid %		
Myristic acid (C14:0)	0.169 ± 0.013 ^a	0.03 ± 0.005 ^a
Palmitic acid (C16:0)	23.054 ± 0.326 ^a	19.298 ± 0.438 ^b
Margaric acid (C17:0)	0.129 ± 0.013 ^a	0.028 ± 0.002 ^a
Stearic acid (C18:0)	6.229 ± 0.286 ^a	3.824 ± 0.164 ^b
Arachidic acid (C20:0)	1.419 ± 0.192 ^a	0.663 ± 0.098 ^a
Palmitoleic acid (C16:1)	0.113 ± 0.015 ^a	0.378 ± 0.023 ^a
Oleic acid (C18:1)	34.934 ± 0.912 ^a	26.929 ± 0.417 ^b
Eicosenoic acid (C20:1)	0.398 ± 0.066 ^a	0.128 ± 0.011 ^a
Linoleic acid (C18:2)	33.05 ± 0.393 ^a	46.41 ± 0.629 ^b
Linolenic acid (C18:3)	0.385 ± 0.146 ^a	1.909 ± 0.480 ^b
Phytosterols %		
Cholesterol	0.364 ± 0.032 ^a	0.91 ± 0.141 ^a
Campesterol	20.276 ± 0.271 ^a	19.401 ± 0.378 ^a
Stigmasterol	6.192 ± 0.229 ^a	6.423 ± 0.698 ^a
β-sitosterol	53.298 ± 0.827 ^a	51.5850.637 ^b
Δ-avenasterol	1.984 ± 0.509 ^a	1.702 ± 0.188 ^a
Δ-7-stigmasterol	1.19 ± 0.262 ^a	1.205 ± 0.096 ^a
Δ-7-avenosterol	0.714 ± 0.036 ^a	0.851 ± 0.067 ^a
Tocopherols %		
α-tocopherol	2.862 ± 0.159 ^a	3.812 ± 0.298 ^b
γ-tocopherol	87.322 ± 0.373 ^a	88.51 ± 0.469 ^b
Δ-tocopherol	2.158 ± 0.285 ^a	0.435 ± 0.020 ^b

^c The values of compounds are expressed as mean ± standard deviation. Values within each row with different superscript letters (a-b) are significantly different at $p \leq 0.05$.

^d Abbreviations are: MPM, Moroccan pearl millet; IPM, Indian pearl millet.

3. Results and discussion

3.1. Proximate analysis

The proximate composition of *P. glaucum* was analyzed using AOAC methods, to assess the nutritional and functional quality of the seeds. The results showed a significant variation between the two cultivars regarding fat, protein, carbohydrate, and energy value (Table 1). Fat content varied from 5 to 7 %, with the highest content observed in the yellow-brown Moroccan variety. Similarly, the mineral content of Moroccan-grown seeds was higher than that of imported seeds. On the other hand, there was little difference between the two varieties in terms of water and dry matter content.

Our findings are consistent with those of other research; the Moroccan variety's fat content is higher than the levels reported by Refs. [16,64–66] from Nigeria, India, Morocco and Cameroon respectively. However, Owhero et al. [67], found the fat content of seeds purchased at the Nigerian Market to be higher than that found in our study. Moisture and dry matter content did not show much difference between the two varieties, but Marmouzi et al. [16], Kulthe et al. [64], and Mawouma et al. [65] reported a higher moisture content ranging from 8 % in Cameroon, 10 % in Morocco, and 12 % in India. As far as ash content is concerned, the yellow-brown variety has a higher ash content than the grayish variety and is close to the 2 % reported by Kulthe and co-authors [64–67]. The fundamental physicochemical characteristics of food, known as functional properties, are determined by the intricate interplay among structural, molecular, compositional, and physicochemical qualities as well as by the environment, conditions of measurement, and associations. Water, oil, swelling capacity, and bulk density are calculated and represented in Table 1. WAC and OAC are crucial parameters to describe the ability of a flour sample to bind to water or oil when the latter are scarce. The results of this study show that Moroccan millet flour absorbs more water and oil than the imported variety, suggesting that it has a high preference for the latter and therefore has more carbohydrates and proteins than millet flour imported from India. The water absorption capacity initially depends on the availability of hydrophilic compounds capable of binding to water

molecules, while the oil's absorption capacity relates to hydrophobic compounds [40,68]. The amount of hydration and swelling capacity of starch granules is measured by their swelling, which also indicates the strength of the associative forces between the crystalline and amorphous phases of the starch grains [69]. The results show a higher swelling capacity in Moroccan flour, which has the consequence that this flour contains more starch, which absorbs much more water expressing the existence of a binding force between the starch particles as well as an indication of the amylose/amylopectin ratio. Bulk density, a gauge of flour heaviness, is a crucial factor in determining which flours are most suited for the packaging and transportation of particle meals. The Indian millet flour sample has the highest bulk density of 0.78 g/cm³, similar to that of Nigeria [41]. This functional property provides information on the condition of a flour sample based on its heaviness and packaging requirements [11]. These variations in the proximal composition and functional properties of the two samples may be due to the physicochemical growing conditions of this species but given the differentiation in seed color and the provenance of the same origin, a link between genetic factors and the fluctuating composition of this plant is created.

3.2. Chemical composition and properties of lipid fraction

3.2.1. Chemical composition

Because of the stressful environments in which they grow, plants evolve defense mechanisms that change the composition of their lipid, protein, or other molecules to preserve normal physiological function and withstand adverse situations [70]. There are already about 400 distinct fatty acids (FAs) identified in the kingdom of plants. Some of these FAs have significant pharmacological action, such as antimicrobial, anti-inflammatory, anti-cancer, and anti-parasitic properties, and some are essential in the prevention of numerous chronic diseases [71, 72]. Due to this, there is a greater need than ever for novel plant-based sources of (FAs) for food preparation and direct ingestion. The results of the chemical composition study of the lipid fraction of two pearl millet cultivars are summarized in Table 2.

Using gas chromatography, we found a significant difference between the two cultivars in terms of oleic, linoleic, linolenic, stearic and palmitic acids. Millet stands out for its high unsaturated fatty acid (UFAs) content, with oleic acid predominating in Moroccan seeds and linolenic acid in those imported from India, while palmitic acid is detected in the third row for both varieties. These results are consistent with those found by Marmouzi et al. [16], where oleic and linoleic acids represent the major FAs in Moroccan *P. glaucum* oil. However, palmitic acid detected a content of 0.63%, lower than that reported by our study for both cultivars. It will therefore be interesting to note that the consumption of diets rich in monounsaturated fatty acids MUFA, especially oleic acid, reduces the prevalence of atherosclerosis, hunger, exerting a protective effect on the β cells of the pancreas regulating the secretion of insulin and the accumulation of neutral lipids, in addition to having effects on the metabolism of lipoprotein [73–75].

In addition to fatty acids, phytosterols are also crucial in maintaining human health. Numerous therapeutic effects, such as hypolipidemia, anti-inflammatory, hypoglycemic, and antioxidant qualities, are displayed by these phytonutrients [76,77]. The two cultivars contained more than 77 % of β-sitosterol, campesterol, and stigmasterol. However, the imported variety is distinguished by the presence of cholesterol, stigmasterol, δ-7-stigmasterol, and δ-7-avenosterol in greater quantity than the other variety. Hence, these results are comparable to those achieved by Ryan et al. [78].

Tocopherol is an important molecule that has powerful pharmacological properties and acts as a radical scavenger, suggesting that it may protect against Alzheimer's disease and cancer [78]. In the present study tocopherols of the two varieties of *P. glaucum* were analyzed and summarized in Table 2. The results of the HPLC analysis show a significant difference between the two cultivars. Three tocopherols were identified, with γ-tocopherol being the major tocopherol in pearl millet seeds.

Table 3

An analysis of the physicochemical properties, chlorophyll content, and carotenoid content of pearl millet seed oils extracted from different sources^{e, f}.

	MPM	IPM
FFA	0.169 ± 0.056 ^a	1.087 ± 0.004 ^a
PV	10.5 ± 0.5 ^a	30.33 ± 1.528 ^b
IV	92.173 ± 0.874 ^a	113.01 ± 2.728 ^b
K ₂₃₂	2.150 ± 0.119 ^a	2.788 ± 0.032 ^a
K ₂₇₀	0.615 ± 0.011 ^a	1.633 ± 0.047 ^a
Chlorophyll	0.213 ± 0.003 ^a	0.255 ± 0.007 ^a
Carotenoid	0.241 ± 0.004 ^a	0.220 ± 0.024 ^a

^e The values of compounds are expressed as mean ± standard deviation. Values within each row with different superscript letters (a-b) are significantly different at $p \leq 0.05$.

^f Abbreviations are: MPM, Moroccan pearl millet; IPM, Indian pearl millet; FFA, Free fatty acid; PV, peroxide value; IV, Iodine value; K₂₃₂, Specific extinction at 232 nm; K₂₇₀, Specific extinctions at 270 nm.

Table 4

Qualitative, Nutritional and Metabolic indexes according FAs profile of *P. glaucum* and *Avena sativa*^g.

	MPM	IPM	PM (Marmouzi et al., 2018)	Oat (Marmouzi et al., 2016)
Qualitative Indexes				
SFA	31	23.841	6.19	19.23
UFA	68.88	75.745	77.36	78.14
PUFA/SFA	1.078	2.026	6.247	1.866
LA/ALA	85.844	24.311	17.77	43.320
UI	102.7	125.982	118.09	114.85
Cox value	3.836	5.461	4.599	4.201
Nutritional Indexes				
NVI	1.785	1.593	68.33	2.911
AI	0.344	0.256	0.0081	0.205
TI	0.831	0.542	0.1205	0.459
hypo/Hyper	2.944	3.893	122.317	4.971
HPI	2.902	3.901	122.793	4.856
Metabolic Indexes				
Elongase	27.019	19.815	739.682	22.048
Δ-9-desaturase 18	84.867	87.565	89.175	92.428
Δ-9-desaturase 16	0.487	1.921	4.545	1.351

^g Abbreviations are: MPM, Moroccan pearl millet; IPM, Indian pearl millet; SFA, Saturated fatty acid; UFA, Unsaturated fatty acid; PUFA, Polyunsaturated fatty acid; LA/ALA, Linoleic acid/ α -linolenic acid; UI, Unsaturation index; Cox, Oxidizability index; NVI, Nutritional value index; AI, Atherogenic index; TI, Thrombogenic index; hypo/Hyper, hypocholesterolemic/hypercholesterolemic fatty acid; HPI, Health promoting index.

Compared to imported pearl millet, which is highly concentrated in γ tocopherol, Moroccan pearl millet stands out for its Δ tocopherol concentration.

3.2.2. Physicochemical properties of lipidic fraction

The determination of food quality is based on content, chemical composition and changes linked to interactions with other components. In fact, lipid degradation through oxidation produces sensory alterations in the product, even if they only make up a small percentage of the product: bad smell, bad taste, discoloration, and texture deterioration [79]. Quality indices for the lipid fraction of the two cultivars of *P. glaucum* are shown in Table 3. The millet seeds have a free fatty acid ranging from 0.16 to 1.08; peroxide values from 10.5 to 30.3 meq O₂/Kg; iodine values from 92.173 to 113.01 g I₂/100 g, and a K₂₃₂ index from 2.15 to 2.78 and K₂₇₀ from 0.61 to 1.63.

The Moroccan cultivar (MPM) appears to have low values for free fatty acid, peroxide value and K₂₃₂ compared to the ranges established for the extra virgin olive oil category (PV < 20; K₂₃₂ < 2.5) as stipulated

in the regulations of the International Olive Oil Council (IOC), which means that this cultivar is of better quality than the imported variety, while the latter may be altered by storage time and method, and even its oxidation status, as demonstrated by UV absorption at 232 nm (formation of primary oxidation products) and 270 (formation of secondary oxidation products). In addition to UV absorption, pigment content provides information on the oxidative stability of the oils, based on their antioxidant activity. The results show a satisfactory carotenoid content for the Moroccan variety (MPM), which prevents peroxidation, unlike the imported variety, which benefits from a fairly high chlorophyll content, leading to peroxidation in the presence of light [35,79]. This phenomenon is mainly linked to the presence of large quantities of polyunsaturated fatty acids in cell membranes, which are then damaged by reactive oxygen species [80]. The iodine value provides information on the degree of unsaturation of lipids, and is defined as the quantity of iodine (g) added to 100 g of oil [81–85]. The results in Table 3 show that the imported variety is characterized by a higher iodine value, reflecting its susceptibility to peroxidation and its high UFA content [86].

3.2.3. Qualitative, nutritional and metabolic indexes

Fatty acids have metabolic properties and can either have a positive or negative impact on human health. Indeed, SFAs are likely to increase the development of coronary heart disease, sclerosis and other diseases related to metabolic syndrome [48,49], unlike polyunsaturated fatty acids, which are recommended in diets for their beneficial effects in the regulation of human metabolism, therefore the evaluation of indices from the lipid profile of millet has been established, thus the various indices were classified according to their functions into qualitative, nutritional and metabolic indices and grouped in Table 4.

Generally, the UFA have the most abundant FAs in the two millet varieties thus marking a high content for the imported variety (IPM, 75.74%) and lower than that found by Marmouzi et al. (77.74%) [16]. The ratio of PUFA to SFA is the most widely used index for assessing the impact of a particular food on cardiovascular health, on the assumption that PUFAs are able to reduce serum cholesterol and LDL-cholesterol, while SFAs increase serum cholesterol levels. According to the results reported in Table 4, the two cultivars showed different values, demonstrating the abundance of PUFAs in millet oil, and specifically in that of the Indian variety. It's worth mentioning that this cereal is beneficial for preventing cardiovascular and chronic diseases and will be highly recommended for human consumption based on this index, since it is higher than 0.4. Among the most dominant PUFAs in millet oil is linoleic acid (C18:2), which has been shown to reduce the growth of atherosclerotic lesions. The relationship between this acid and linolenic acid (C18:3), which cannot be synthesized in the mammalian body and must be supplied through diet, was investigated. In the light of the results shown in Table 4, both cultivars are characterized by a high level of this ratio, with the remarkable proportion in the Moroccan millet, due to the high linoleic acid content of pearl millet. By lowering blood cholesterol, LDL-cholesterol, and the ratio of total cholesterol to HDL-cholesterol, a diet high in n-6 PUFAs can lower the risk of cardiovascular disease [87, 88]. The unsaturation index (UI) indicates the degree of unsaturation of lipids, it makes it possible to highlight the role of PUFAs and MUFAs at the same time contrary to the ratio PUFA/SFA [53]. In the present study, millet grown in Morocco had a value of 102.7 less than that of the imported variety indicating a lower risk of auto-oxidation of FAs, thus confirming the results of the quality indices (PV; IV; K₂₃₂) presented in Table 3 and the quality of Moroccan seeds. Similarly, the calculated oxidizability value (Cox) indicates a higher oxidation resistance for MPM due to its low content by comparing it with the imported one and those reported by Marmouzi et al. [16,89]. This value is calculated on the basis of the percentages of UFAs and allows us to estimate the ability of an oil to oxidize. However, the nutritional indices record high values for Moroccan *P. glaucum* (MPM) for nutritional value index (NVI), atherogenicity (AI), and thrombogenicity (TI), in contrast to the Hypo/Hyper Ratio and the Health Promotion Index (HPI). Thus, the

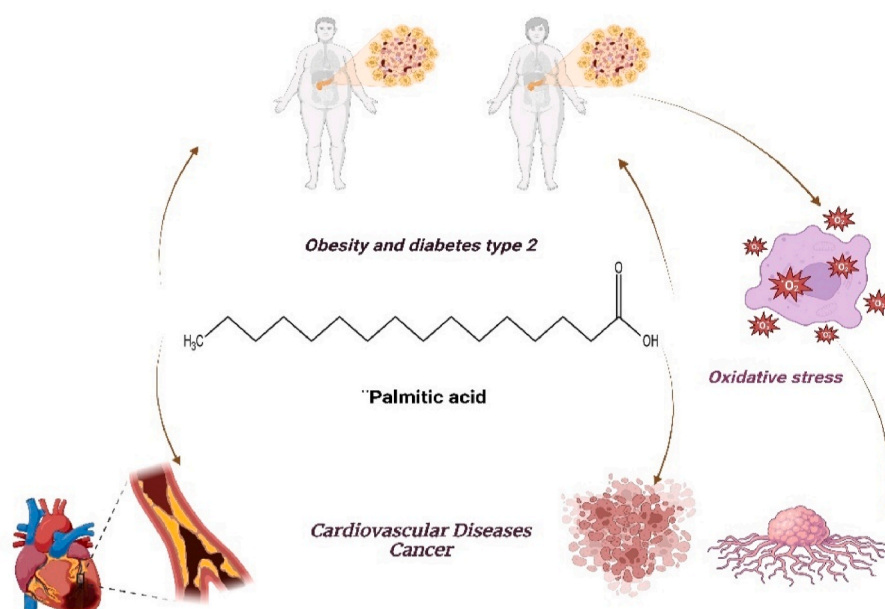


Fig. 1. Palmitic acid and human health.

highest value of NVI is the result of the high proportion of oleic and palmitic acid, while the value marked by Marmouzi et al. [16] is mainly due to the low proportion of C16:0 palmitic acid. Other than NVI, the atherogenicity index (AI) highlights the link with the main proatherogenic fatty acids (SFAs) and antiatherogenic ones (UFAs), the results marked on Table 4 indicate a low value for seeds imported from India (IPM), and this is due to its richness in UFAs. Consequently, this index decreases with increasing UFA content in the oils, and this implies that a low AI value will be beneficial to health, since UFAs are able to inhibit plaque aggregation and reduce cholesterol levels [90,91]. Similarly, to AI, the thrombogenicity index decreases with increasing UFAs content, indicating that a low TI is beneficial to the nutritional quality of a food. In fact, this index highlights the connection among prothrombogenic (SFAs) and antithrombogenic (UFAs) fatty acids. Briefly, TI and AI are nutritional and pharmaceutical indices, which enable us to determine the effects of chemical FAs composition on cardiovascular health. We can therefore deduce that low AI and TI represent better nutritional quality, and that consumption of food products based on these two indices reduces the risk of coronary heart disease [48]. In contrast to these last two indices, a high value of the hypo/Hyper ratio is desirable to increase the nutritional quality of food [87,92]. In the present study, the hypo/Hyper ratio varies between 2.94 and 3.89 for the two studied cultivars of millet, while Marmouzi et al. [16,89] found a higher value (4.971 and 122.317) of this ratio in pearl millet which is desirable for good cardiovascular health. This discrepancy between the different varieties is due to the difference in C16:0 palmitic acid content. At the same time, the health promotion index represents a big difference between millet varieties and oats. Overall, the nutritional indices show a good quality of food provided that UFAs represent most FAs, as well as a low content of palmitic acid. The long-chain FA palmitic acid is either produced internally from an excess of energy from proteins or carbohydrates or it can be obtained from diet. It is linked to the emergence of numerous metabolic syndrome-related illnesses Fig. 1 [93,94].

At the molecular interface, PUFAs play a vital role in human health, through their involvement in various biochemical functions (synthesis of inflammatory mediators, gene expression, membrane fluidity and intracellular regulation), as well as their action in specialized cells (liver, heart, brain and retina), due mainly to long-chain PUFAs (C18:2; C18:3 ...) which are correlated with several pathologies linked to metabolic disorders, Alzheimer's disease, and Crohn's disease [95]. In the

	C16:0	C18:1	FFA	PV	IV	K232	K270	UI	Cox	NVI	IA	IT	h/H	HPI
C16:0	1.00	0.98	-0.98	-0.99	-0.99	-0.96	-0.99	-1.00	0.87	1.00	1.00	-1.00	-1.00	-1.00
C18:1	0.98	1.00	-1.00	-0.98	-0.98	-0.99	-0.99	-0.98	-0.98	0.96	0.97	0.97	-0.97	-0.97
FFA	-0.98	-1.00	1.00	0.99	0.98	0.99	1.00	0.99	0.99	-0.94	-0.98	-0.98	0.98	0.98
PV	-0.99	-0.98	0.99	1.00	0.98	0.97	1.00	1.00	1.00	-0.89	-1.00	-0.99	0.99	0.99
IV	-0.99	-0.98	0.98	0.98	1.00	0.95	0.98	0.99	0.99	-0.90	-0.99	-0.99	0.99	0.99
K232	-0.96	-0.99	0.99	0.97	0.95	1.00	0.98	0.97	0.97	-0.94	-0.96	-0.95	0.96	0.96
K270	-0.99	-0.99	1.00	1.00	0.98	0.98	1.00	0.99	0.99	-0.92	-0.99	-0.99	0.98	0.99
UI	-0.99	-0.98	0.99	1.00	0.99	0.97	0.99	1.00	1.00	-0.88	-1.00	-1.00	1.00	1.00
Cox	-1.00	-0.98	0.99	1.00	0.99	0.97	0.99	1.00	1.00	-0.89	-1.00	-1.00	1.00	1.00
NVI	0.87	0.96	-0.94	-0.89	-0.90	-0.94	-0.92	-0.88	-0.89	1.00	0.87	0.87	-0.85	-0.86
IA	1.00	0.97	-0.98	-1.00	-0.99	-0.96	-0.99	-1.00	-1.00	0.87	1.00	1.00	-1.00	-1.00
IT	1.00	0.97	-0.98	-0.99	-0.99	-0.95	-0.99	-1.00	-1.00	0.87	1.00	1.00	-1.00	-1.00
h/H	-1.00	-0.97	0.98	0.99	0.99	0.96	0.98	1.00	1.00	-0.85	-1.00	-1.00	1.00	1.00
HPI	-1.00	-0.97	0.98	0.99	0.99	0.96	0.99	1.00	1.00	-0.86	-1.00	-1.00	1.00	1.00

Fig. 2. *Pennisetum glaucum*'s lipidic fraction's qualitative and nutritional indices and physico-chemical parameters are correlated according to Pearson's correlation coefficient.

organism, PUFAs are converted by enzymes (elongase, desaturase and Acyl CoA synthetase) into very long-chain PUFAs. An example is α -linolenic acid C18:3, which promotes the production of eicosanoids with an anti-inflammatory effect by the enzyme's desaturase and elongase. For this reason, millet seems to have these 2 key enzymes for transforming α -linolenic and linoleic acid [96].

To explore the relation between qualitative and nutritional indexes and phytochemical content for *P. glaucum* oil, the correlation coefficient of the proportion of C16:0, C18:1; quality and fatty acid indexes are shown in Fig. 2.

Quality indices for the lipid fraction of pearl millet (FFA, PV, IV, K₂₃₂, K₂₇₀) showed a positive correlation with FA quality indexes (UI and Cox) and with nutritional indexes (hypo/Hyper and HPI). These indices tell us about the degree of unsaturation by IV similar to the unsaturation index (UI); and the degree of oxidation by PV, K₂₃₂, K₂₇₀ identical to the calculated oxidizability (Cox). Palmitic and oleic acid contents were also positively correlated with the AI, TI and NVI millet nutrient values, in contrast to the hypo/Hyper and HPI ratios, which were strongly negatively correlated, demonstrating that an increase in palmitic A. content leads to a decrease in the hypo/Hyper ratio, meaning an increase in serum cholesterol because of the increase in

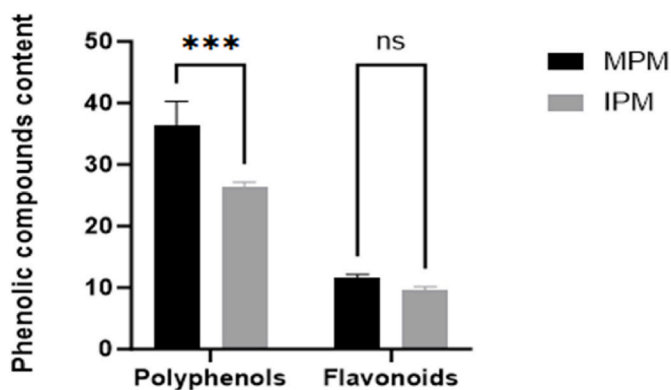


Fig. 3. Polyphenols (mg GAE/g extract) and Flavonoids (mg EQ/g extract) content in *Pennisetum glaucum* extract. Data was analyzed by two-way ANOVA. Data with *** were statistically different at $p \leq 0.001$ by Sidak's multiple comparisons test.

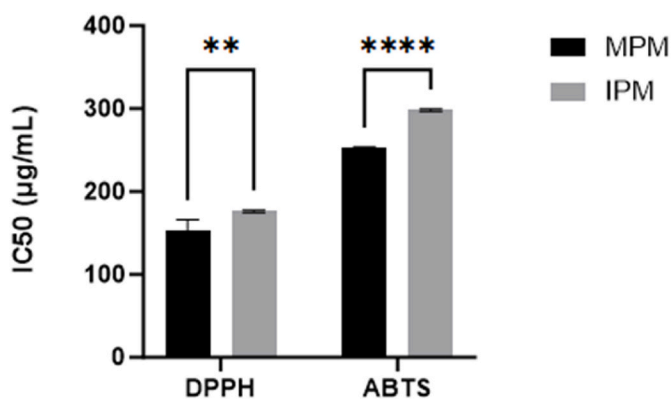


Fig. 4. Antioxidant activity of two cultivars pearl millet (DPPH and ABTS). Data was analyzed by two-way ANOVA. Data with ** were statistically different at $p \leq 0.01$ and those with **** were statistically different at $p \leq 0.001$ by Sidak's multiple comparisons test.

hypercholesterolemic agent (SFAs).

3.3. Polyphenols and flavonoids content

The most prevalent secondary metabolites in plants are phenolic compounds, which are distinguished by the presence of one or more hydroxyl groups and by their antioxidant qualities, which help to prevent damage from free radicals [97,98]. The analysis of the polar fraction of *P. glaucum* seeds allowed to quantify their polyphenols and flavonoids contents cited in Fig. 3.

Based on the results shown in Fig. 3, a significant variation was recorded between the two cultivars of *P. glaucum* polyphenols content. Phenolic compound content is more concentrated in the Moroccan-grown variety (MPM) than in the imported variety (IPM). In fact, total polyphenol content varied between 26.380 and 36.278 mg GAE/g extract, higher than the values found by Marmouzi and co-authors when comparing the two Moroccan varieties [16]. However, the Indian variety represents a low content, as also demonstrated by Salar et al. [14], who determined a value of 7.32 mg GAE/g extract to be the highest among the different genotypes studied. In addition to polyphenols, flavonoids are one of the main pigments in plants, giving them great color variation [99]. As a result, differentiation in the content of this class of secondary metabolites may be the main cause of the color variation in the seeds of these two varieties of *P. glaucum*, where the Moroccan variety is found to have a higher content of 11.580 mg EQ/g extract than the imported variety with a value of 9.736 mg EQ/g extract. These

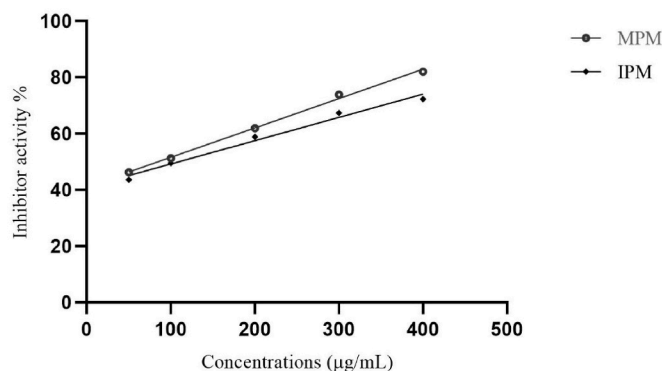


Fig. 5. α -amylase inhibitor activity % of two cultivars of *Pennisetum glaucum*.

results are higher than those found by Mawouma et al. [65].

3.4. Antioxidant activity

Antioxidant bioactive compounds are becoming more and more valuable in the twenty-first century due to their special functions as lipid stabilizers and suppressors of unneeded oxidative stress, which is linked to the development of cancer and aging. Antioxidant activity has been shown to occur in a variety of ways, including as the inhibition of oxidizing enzymes, the chelating agents of transition metals, the deactivation of single oxygen atoms, the transfer of hydrogen or a single electron to radicals, and the enzyme-mediated detoxification of reactive oxygen species. There are a number of procedures that are commonly used in order to evaluate the antioxidant properties of secondary metabolites and natural extracts. ABTS and DPPH are examples of such procedures. This method relies on a spectrophotometer that is capable of detecting color changes caused by radical saturation. The results of DPPH and ABTS radical scavenging activity were indicated in Fig. 4 as compared with a positive standard of Trolox. We may conclude that, in comparison to the standard, the scavenging effects of these millet variety extracts on DPPH radicals and ABTS were not as noteworthy. In addition, the Moroccan varieties of pearl millet had more inhibition than the Indian counterparts. When compared to other grains, pearl millet generally showed low antioxidant activity [65,100].

3.5. Antidiabetic activity

3.5.1. α -amylase inhibition test

The amylase enzyme hydrolyzes starch molecules, yielding a variety of products including dextrans and progressively smaller polymers made up of glucose molecules [101]. As a hydrolase enzyme, α -amylase catalyzes the hydrolysis of internal α -1,4-glycosidic linkages in starch to produce products such as glucose and maltose [102]. Fig. 5 summarizes the inhibitory activity (%) of two cultivars of *Pennisetum glaucum* on α -amylase activity.

It was found that the amount of inhibition increased with extract concentration. In contrast to the imported millet, Moroccan millet demonstrated strong inhibition with an IC₅₀ of $84,439 \pm 1.245$ µg/mL compared with $109,02 \pm 3.156$ µg/mL for the imported millet. It is acknowledged that initial hypoglycemic agents such as carbolytic enzyme inhibitors (Acarbose) are clinically useful in controlling hyperglycemia, however, we relied on their use as a standard intervention. As a result, when comparing the different IC₅₀ values, acarbose (32.41 ± 0.05 µg/mL) performs better than our extracts in terms of hypoglycemic activity. Based on their study on alpha-amylase catalytic inhibitory capacity on a set of genotypes from different climatic zones of India, Krishnan et al. [21] recorded an IC₅₀ that ranged from 73.06 to 104.2 µg/mL which are consistent with those of our study. In alternative studies, Nani et al. [103] examined the anti-diabetic potential of pearl

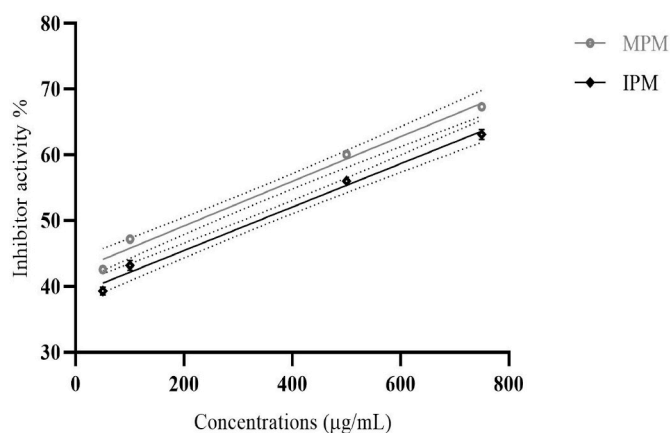


Fig. 6. α -glucosidase inhibitor activity % of two cultivars of *Pennisetum glaucum*.

millet by inducing diabetes with Streptozotocin, and found changes in carbohydrate metabolism following a diet rich in millet. A key component of the inhibitory activity of α -amylase is polyphenols, so Sun et al. [104] have demonstrated that for optimum inhibition of the enzyme, phenolic compounds should carry hydroxyl groups in their molecular structure, as inhibition probably depends upon hydrogen bond formation between the $-OH$ groups of phenolic compounds and amino acid side chains on the active site of α -amylase.

3.5.2. α -glucosidase inhibition test

An α -glucosidase inhibitor is a family of pharmaceuticals used to treat type 2 diabetes, either alone or in conjunction with other anti-diabetic medications, these inhibitors limit α -glucosidase action in the small intestine, preventing carbs from being digested there. Their mode of action involves inhibiting enzymes that transform complicated non-absorbable carbs into simple carbohydrates that are easily absorbed [105]. Fig. 6 summarizes the inhibitory activity (%) of two cultivars of *P. glaucum* on α -glucosidase activity. There was a linear relationship between the percentage of inhibition and the concentrations of alcoholic extracts of the two pearl millet cultivars. When comparing the two cultivars, Moroccan millet had the most inhibitory effect, with a lower mean IC_{50} value of 224.103 μ g/mL than Indian millet ($IC_{50} = 337.818$ μ g/mL). When compared to the standard ($IC_{50} = 78.893$ μ g/mL), extracts had the lowest alpha glucosidase inhibitory activity. Previous investigations found that polyphenol-enriched millet extracts had IC_{50} values ranging from 73.80 to 103 μ g/mL higher inhibitory effect than our study.

3.6. Correlation analysis

To investigate the impact of the protein, total polyphenols, and total flavonoids content on the antioxidant and antidiabetic properties of *P. glaucum*, the correlation coefficients were assessed Table 5.

The findings revealed a high positive correlation between the various bioactive components of pearl millet (protein, TPC, and TFC). These

Table 5

Pearson's correlation coefficient between phytochemical components and bioactivities of pearl millet extracts (IC_{50})^h.

	Protein	TPC	TFC	DPPH	ABTS	α -amylase	α -glucosidase
Protein	1						
TPC	0.894	1					
TFC	0.945	0.976	1				
DPPH	-0.883	-0.998	-0.964	1			
ABTS	-0.991	-0.872	-0.910	0.866	1		
α -amylase	-0.990	-0.912	-0.935	0.909	0.995	1	
α -glucosidase	-0.991	-0.866	-0.907	0.860	0.999	0.994	1

^h Value in bold are different from 0 at significance level $\alpha = 0.05$.

bioactive chemicals have a high negative connection with the activities evaluated. Indeed, millet proteins revealed to have an action on the IC_{50} of ABTS ($r = -0.991$) and α -glucosidase ($r = -0.991$), implying that increasing protein concentration will reduce the inhibitory concentration (IC_{50}), hence enhancing millet's antioxidant and anti-diabetic properties. Previous research has established the bioactivity of dietary proteins not only as antioxidants and anti-diabetics [33,106], but also as antibacterial, anti-inflammatory, anticancer, and antihypertensive agents [107]. Additionally, it appears that phenolic compounds, such as polyphenols and flavonoids, have a significant impact on α -amylase and DPPH. A strong negative correlation indicates that an increase in these compounds' content will lower the IC_{50} against α amylase and DPPH and strengthen the activities under investigation. Indeed, polyphenols can relieve type 2 of diabetes symptoms by suppressing disaccharidases in the intestinal lumen, which limits polysaccharide breakdown and reduces sugar absorption. They also have anti-diabetic properties via increasing glucose absorption in muscle and adipocytes [108]. The glycosides of kaempferol and quercetin have a significant role in inhibiting enzyme activity [109]. However, there is a substantial positive association between both activities, which suggests that oxidative stress and diabetes are closely related. This has been shown in several research. A study conducted by Hasan et al. [110] revealed a good link between the reference antioxidant ascorbic acid and the inhibition of α -glucosidase by jujube extract. Additionally, further research has shown the ability of antioxidants to integrate at the insulin level [111].

4. Conclusion

In this study, we present a qualitative and quantitative analysis of the nutritional, functional, and pharmacological properties of *P. glaucum*, starting from the proximal analysis of the seeds, subsequently delipidated, and from the examination of the various compounds with nutritional and medicinal properties, to finally arrive to the polar part, where phenolic compounds that have anti-diabetic and antioxidant properties are found. Research has demonstrated the nutritional value of pearl millet by highlighting its abundance of primary and secondary metabolites, such as proteins, minerals, carbohydrates, fatty acids, sterols, tocopherols, polyphenols, and flavonoids. Furthermore, the study highlighted the pharmacological potential of the seed by comparing the ratio of fatty acids that lower cholesterol to those that raise cholesterol, as well as its thrombogenicity and atherogenicity indexes, which suggest that this seed can be included in diets to prevent cardiovascular disease. Finally, the α amylase inhibitory activity of the seeds was moderate compared to acarbose. Overall, *P. glaucum* is a plant that possesses the advantageous qualities of being both a source of nutrition and medicine.

CRedit authorship contribution statement

Chaimae El Kourchi: Formal analysis, Data curation, Conceptualization. **Oumayma Belhoussaine:** Validation, Software, Methodology. **Hamza Elhrech:** Visualization, Validation, Methodology, Formal analysis. **Hicham Harhar:** Software, Project administration, Methodology, Investigation. **Riaz Ullah:** Conceptualization, Data curation, Writing –

review & editing. **Ahmed Bari:** Conceptualization, Data curation, Formal analysis. **Filippo Maggi:** Writing – review & editing, Supervision, Software. **Giovanni Caprioli:** Writing – review & editing, Supervision. **Abdelhakim Bouyahya:** Writing – review & editing, Supervision, Project administration. **Mohamed Tabyaou:** Writing – review & editing, Visualization, Validation, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors are unable or have chosen not to specify which data has been used.

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