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Nutritional potential, phytochemical analysis, and biological activities of quinoa (*Chenopodium quinoa* Willd.) seeds from arid zone culture

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Abstract

Quinoa, scientifically known as *Chenopodium quinoa* Willd., is well recognized for its exceptional nutritional composition and potential benefits for human health. This research aims to conduct a phytochemical analysis of nutraceutical properties and biologcal activities of seven different quinoa cultivars, namely, red, white, *Amarilla marangani*, kancolla, Giza 02, *Amarilla sacaca*, and black. The analysis of ground seeds revealed that Giza 02, white, and *A. marangani* had the most significant protein, fat, ash, and carbohydrates, respectively. Furthermore, the MeOH extracts of the various quinoa genotypes exhibited high levels of total phenolic, flavonoid, condensed tannin and reducing sugar contents in the seed extracts obtained using consecutive solvents. In addition, HPLC-DAD analysis revealed the presence of seven phenolic compounds, which may be classified into four families: phenolic acid, flavonoids, coumarins, and stilbenes. The IC_{50} values for the antioxidant activity ranged from 6.4 to 7.7 µg/mL. Furthermore, the variability in the antibacterial activity across different genotypes and gram-positive and gram-negative bacterial species was observed, and the efficacy of the CYHA-red extract against *Micrococcus luteus* was the highest. The findings of this study indicate that the incorporation of quinoa into the arid and Saharan cultivation system has the potential to enhance the nutritional and nutraceutical characteristics of the seeds.

Keywords: Quinoa seeds; nutritional value; phytochemicals; biological activities; multivariate analysis.

Introduction

The plant known as quinoa (*Chenopodium quinoa* willd.) belongs to the Amaranthaceous family and is classified as an annual herbaceous plant. The origin of this species is from the Andean area of South America, and it underwent domestication by indigenous populations many millennia ago (Schmidt *et al.,* 2023). Over time, the plant has

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undergone adaptations to thrive in challenging soil conditions and harsh ecological circumstances (Hussin *et al.,* 2023). Quinoa is classified as a pseudo-cereal crop due to its broad-leaf nature and starchy dicotyledonous seeds, categorizing it as a noncereal crop (Nisar *et al.,* 2017). Quinoa has inherent resilience to dry soils, and the FAO sees its cultivation as a means to combat famines (Karthika and Govintharaj, 2022). Compared to wheat, maize, barley, rice, and oats, quinoa has a notably higher protein content, calcium, magnesium, potassium, iron, and zinc, as well as vitamins A and E. Moreover, it should be noted that quinoa is the only edible plant that offers the primary amino acids (Chaudhary *et al.,* 2023; Pirzadah *et al.,* 2023). In contemporary times, several scholars have lately surfaced to investigate the chemical components included in quinoa seeds and their potential therapeutic attributes. This crop has been recognized as a significant asset in the advancement of functional foods, which provide distinct health advantages (Balakrishnan and Schneider, 2022; Brito *et al.,* 2022; Yang *et al.,* 2022). Apart from the beneficial effects on human health resulting from the ingestion of seeds, some bioactive chemicals have shown intriguing pharmacological characteristics, indicating potential uses within the pharmaceutical domain (Afzal *et al.,* 2023; Shah *et al.,* 2022). Quinoa is classified as a gluten-free meal, and research has shown that consistent intake of quinoa enhances the condition of the small intestine in individuals with celiac disease, facilitating a more rapid restoration of their intestinal villi than a basic gluten-free diet (Brito *et al.,* 2022). However, research is scarce on the nutritional composition, antioxidant properties, and biological attributes of quinoa grown in Algeria. Hence, the analysis of the biochemical makeup of quinoa seeds has garnered attention owing to their longstanding use as a regional food item, particularly in the southern regions of Algeria. As a result, the study was conducted to examine the chemical composition (total phenolic content (TPC), total flavonoids content (TFC), condensed tannin content (CTC), reducing sugar content (RSC)) and to identify the phenolic compounds using a high-performance liquid chromatography-diode array detector (HPLC-DAD) and to determine the biological activity (antioxidant and antibacterial) of seven quinoa genotype extracts.

Materials and Methods

Chemical used

All chemicals used were of analytical reagent grade. All reagents were purchased from Sigma, Aldrich (France): Acetic acid, ACN, DMSO, bovine serum albumin (BSA), cyclohexane (CYHA), dichloromethane (DCM), methanol (MeOH), Folin–Ciocalteu reagent (2N), gallic acid (GA), quercetin(Q), catechin(C) (purity > 98%), HCl, and EtOH.

Plant material

The seeds of seven cultivars of quinoa (*Chenopodium quinoa* Willd.) were used in this study. These varieties were introduced to Algeria in 2014 as part of the quinoa crop dissemination project implemented by the Food and Agriculture Organization of the United Nations (FAO). They were grown in an open field measuring 1,000 square meters during the 2019/2020 season in Oued Souf region, Algeria. All the varieties were obtained from Egypt (*Amarilla marangani*, *Amarilla sacaca*, white, Giza02, and kancolla), and Peru (black and red) through the Technical Institute for the Development of Desert Agriculture (TIDDA).

Macronutrients analysis

The samples were analyzed for chemical composition (total fat, total proteins, carbohydrates, and ash) using the AOAC procedures (Horwitz *et al.,* 1975). Total protein content was analyzed using the Bradford as a reagent and bovine serum albumin (BSA) as a standard. Total fat content was determined by extracting a known weight of sample powder with CYHA using a cold extraction. The ash content was determined after incineration of the sample crude at 550°C for 8 hours. The total carbohydrates were calculated by difference of mean values according to the following equation (Eq. (1)):

$$\text{Carbohydrate} = [\text{total solid} - (\text{protein} + \text{lipids} + \text{minerals})]. \quad (1)$$

Biochemical analysis

Extracts preparation

A mass of 10 g of crude material of each genotype was successively extracted with a constant solid/liquid ratio of 1:10 ($w\ v^{-1}$) for 2 h using different solvents of increasing polarity, namely, CYHA, DCM, and MeOH. After each extraction, the extract was filtered through filter paper, and the solvent was removed using a rotary vacuum evaporator R-200 (Buchi Labortechnik) under pressure at 35°C. The obtained dry residues were put in hemolysis tubes and stored at −20°C until their use. After that, the yield extraction was calculated according to the following equation (Eq. (2)):

$$\text{Yield (\%)} = (m / M) \times 100, \quad (2)$$

with m being the weight of dry extract (g), and M being the weight of seeds material (g).

Determination of TPC

The TPC was determined spectrophotometrically using the Folin–Cioclateu method (Sassi Aydi *et al.,* 2020). Briefly, 100 µL of sample extract was mixed with 500 µL of 10-fold diluted Folin–Ciocalteu's phenol reagent and allowed to react for 5 min. After that, 400 µL of Na_2CO_3 solution (7%) was added, and the final volume was brought to 2 mL with distilled water. After 1 h at room temperature, the absorbance at 760 nm was measured using a T80 UV–Vis spectrophotometer. A calibration curve was prepared using a positive control of gallic acid (GA). Results were expressed as mg GAE/g of the dr.

Determination of TFC

The total flavonoid content was determined using a colorimetric method used by Ben Khadher *et al.* (2022), with minor modifications. An aliquot (500 µL) of each quinoa extract was added to 1.5 mL distilled water, 150 µL of sodium nitrite solution (5%,), and mixed for 6 min before the addition of 150 µL 10% aluminum chloride. The volume was adjusted with distilled water to 2.5 mL, and then incubated at room temperature for 6 min. The reaction was completed by adding 500 µL of sodium hydroxide (4%). The final absorbance was determined at 510 nm against a blank. The TFC was reported as mg QE/g dr against the calibration curve of quercetin (Q).

Determination of CTC

CTC was assayed using the method previously described by Sassi Aydi *et al.* (2020). The seed extracts (50 µL) were added to 3 mL of 4% vanillin (w/v in MeOH) and 1.5 mL of concentrated HCl. After that, the absorbance was measured at 500 nm after incubation in the dark, for 15 min, at room temperature. The condensed tannins content in the different quinoa extracts was expressed as mg CE/g dr.

Determination of RSC

The reducing sugar content of different quinoa extracts was determined using the 3,5-dinitro salicylic acid (DNSA) method. The measurement was performed according to the procedure used by Ayadi *et al.* (2023), with slight modifications. DNSA reagent was prepared by dissolving 1 g of DNSA and 30 g of sodium-potassium tartaric acid in 80 mL of 0.5 N NaOH at 45°C. After dissolution, the solution was cooled to room temperature and diluted to 100 mL with distilled water. For the measurement, 2 mL of DNSA reagent was pipetted into a test tube containing 1 mL of plant extract (1 mg/mL) and kept at 95°C for 5 min. After cooling, 7 mL of distilled water was added to the solution, and the resulting solution's absorbance was measured at 540 nm using a UV-VIS spectrophotometer. The RSC was calculated using the calibration curve of standard D-glucose (200–1,000 mg/L), and the results were expressed as mg glucose equivalent (GE) per gram dry extract weight.

HPLC-DAD analysis

The HPLC analysis was done with an HPLC-DAD system (Agilent Technologies) equipped with a quaternary pump (1260 Quat Pump VL) and a UV–Vis detector (1260 DAD VL) according to the method used by Saoudi *et al.* (2021), with some modifications. At room temperature, the separation was carried out on a column (RPC18, 25 cm × 4.6 mm and a particle size = 5 µm). Elution was performed at a flow rate of 1.2 mL/min, using a mobile phase consisting of acidified water (pH2.65) (solvent A) and acidified water/ACN (20:80 v/v) (solvent B). The following linear gradient eluted the samples: from 0.1% B to 30% B for 25 min, from 30% B to50% B for 5 min, from 50% B to 99.9% B for 10 min, and finally from 99.9% B to 0.1% B for 15 min. The total acquisition time was 55 min. A volume of 20 µL of each sample was injected, and the detection was done at 280 nm. The different organic extracts were prepared at a concentration of 20 mg/mL (80:20 v/v) acidified water/ACN, then filtered through a micro-filter (Millex-HA filter 0.45 µm). The phenolic compounds were identified by comparison of the retention time of some known standards and then quantified using their calibration curves.

2,2-Diphenyl-1-picrylhydrazyl radical scavenging assay

The antioxidant activity of samples was determined using the DPPH method described by Sassi Aydi *et al.* (2020), with minor modifications. An aliquot of 500 µL of extract in known concentrations (0–5mg/mL) was mixed with 500 µL of DPPH solution (2 mg in 100 mL ethanol). The reaction was incubated for 1h in the dark at room temperature; temperature and the absorbance were measured at 517 nm using a spectrophotometer. The blank was prepared for each concentration without DPPH solution. Ascorbic acid was used as a positive control (standard). The inhibition percentage was calculated as follows:

$$\text{DPPH radical scavenging activity I(\%)} = [(A_{blank} - A_{sample})/A_{sample}] \times 100.$$

Antibacterial activity

The quinoa seed extracts were tested individually against four human-pathogenic microbial strains: two

gram-positive bacteria (*Listeria monocytogenes* and *Micrococcus luteus*) and two gram-negative ones (*Escherichia coli* and *Salmonella typhimurium*). The bacteria used were selected for their involvement in the human skin, and oral and intestinal tracts. The antimicrobial activities of quinoa seed extracts were assayed using the suitable diffusion method, according to Sassi Aydi *et al.* (2020). Antibacterial activity was checked by adding 50 µL of a solution at 100 µg/mL concentration to three wells. An additional well in each plate was filled with solvent 2% DMSO as a negative control, while ampicillin (5 µg/mL) was used as a positive control. Allow the culture plate to spread for 2 h at room temperature. Plates were then incubated at 37°C upright for 24 h.

Statistical analysis

All data were expressed as means ± standard deviations of triplicate measurements. The confidence limits were set at $p < 0.05$ and calculated according to the ANOVA test using the Statistical Package for the Social Sciences (SPSS) 22 (Version IBM. 22.0. 2013, San Francisco, CA, USA, www.ibm.com). Tukey's test estimated the difference between the genotypes. Principal component analysis (PCA) was also conducted using XLSTAT (version 2021.3.1, Addinsoft, Pearson edition, Waltham, MA, USA).

Results and discussion

Nutritional value

As shown in Table 1, there was considerable variation in the nutritional value between the seven quinoa seed samples. The total protein content of the varieties analyzed ranged from 11.0 g/100 g for red quinoa to 25.9 g/100 g for Giza 02, with an average of 20.5 g/100 g, which was in line with that found for quinoa at 25.5 g/100 g by Sánchez-García *et al.* (2022). These current results were higher than the values reported in the study of Li *et al.* (2021), when working on quinoa seeds from the United Kingdom. Fat contents found in the different quinoa genotypes were comparable, with an average of 3.6 g/100 g, except for red and white quinoa. While white quinoa exhibited the highest total fat, the red one showed the lowest value (Table 1).

The last study by Gómez *et al.* (2021) showed a close fat value enregistrate in pot-4 quinoa. In addition, almost all quinoa varieties showed comparable ash content. The average of this parameter (3.0 g/100 g) found in the present study was practically 1.5-fold higher than the value found by Villacrés *et al.* (2022) in their work on quinoa grains. Depending on the degree of polymerization, carbohydrates can be classified as monosaccharides, disaccharides, oligosaccharides, and polysaccharides (starches). In the present study, carbohydrate content in all substrates was more than 65 g/100 g, which is in the range of multiple quinoa varieties recorded by Hussain *et al.* (2021) in their work in the order of 65 g/100 g. The exception was highlighted by A. marangani, and kancolla showed the highest amount of carbohydrates with 82.4 and 81.4 g/100 g, respectively (Table 1).

Phytochemical composition

Extraction yield, TPC, TFC, and CTC

The solvent efficiency used to extract specific components from the raw material determines the extraction yield. Table 2 showed the yield obtained from different extracts. The current results showed that the maximum percent yield was obtained when the other plant material of all the varieties of quinoa was extracted with MeOH, followed by CYHA, and finally by DCM. The only exception was found for the CYHA-red extract, with a low amount of total extractable compounds compared to the other CYHA ones (0.7%). This result showed that the most extractible compounds were fatty acids soluble in the apolar solvent (CYHA) or phenolics soluble in a polar solvent (MeOH).

As shown in Figure 1, the TPC differed significantly among kancolla and *A. sacaca* compared to black and red quinoa ($p \leq 0.05$). The statistical results for the other species showed no significant difference between them ($p > 0.05$). The TPC in the different quinoa seeds ranged from 210.1 to 720.6 mg GAE/100 g dr, for DCM-*S. sacaca* and CYHA-black, respectively. While the MeOH extracts

Table 1. Physicochemical composition of the different quinoa genotypes (gram/100 g dry weight).

Parameters	Red	White	Amarillamarangani	Kancolla	Giza 02	Amarilla sacaca	Black
Protein	24.5	20.3	11	11.9	25.9	25	25.6
Fat	0.7	4.7	3.7	3.8	3.1	3.6	3.6
Ash	3.2	3.5	3	3.2	2.9	2.9	2.4
Carbohydrates	72.1	71.8	82.4	81.4	68.1	68.5	68.4

Table 2. Extraction yield of various quinoa genotypes organic extracts.

Species/ solvents	Yield (%)		
	CYHA	DCM	MeOH
Red	0.7	0.5	3.5
White	4.7	1.0	5.8
Amarilla marangani	3.7	0.7	6.8
Kancolla	3.8	0.9	7.6
Giza 02	3.1	0.9	8.0
Amarilla sacaca	3.6	0.8	5.2
Black	3.6	0.7	4.6

CYHA: cyclohexane; DCM: dichloromethane; MeOH: methanol.

of the majority of species (red, kancolla, Giza 02, and *Amarilla acacia)* exhibited *the* highest TPC, the DCM-*A. marangani*, CYHA-white, as well as CYHA-black showed the highest contents (Figure 1).

Our results agreed with the previous finding of Liu *M et al.* (2020), when working on MeOH extracts of white, red, and black quinoa. Moreover, the actual results were confirmed by the work of Melini *V. and Melini F.* (2021). They mentioned that the TPC ranged between 873-994, 410-1152, and 623-1131 mg GAE/100 g dr for red, white, and black quinoa, respectively. Concerning the TFC, the MeOH extracts of all the quinoa species exhibited the highest amounts compared to DCM and CYHA solvents (Figure 2).

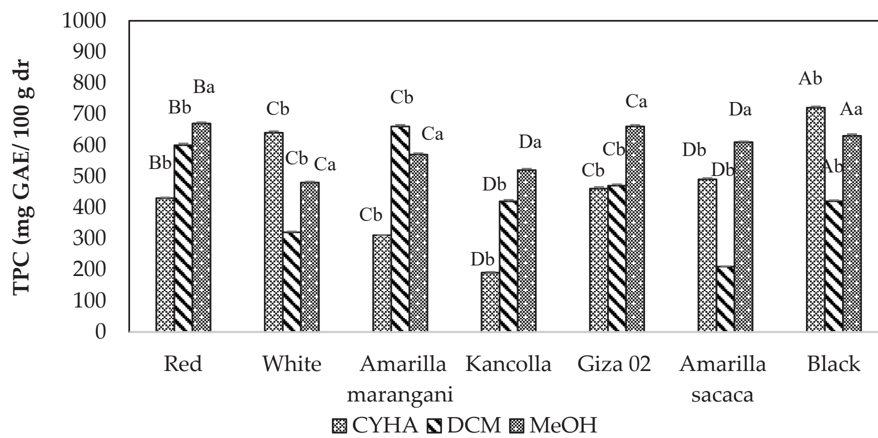

Figure 1. Total phenolic content in the different extracts of quinoa genotypes. CYHA: cyclohexane; DCM: dichloromethane; MeOH: methanol. Different letters on the histograms mean a significant difference; capital letter means the difference between organs; lowercase letter means difference between solvents (p ≤ 0.05).

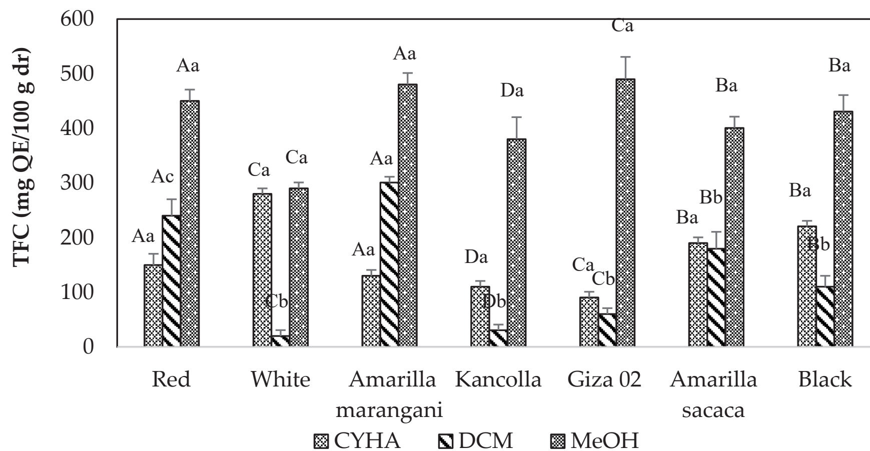

Figure 2. Total flavonoid content in the different extracts of quinoa genotypes. CYHA: cyclohexane; DCM: dichloromethane; MeOH: methanol). Different letters on the histograms mean a significant difference; capital letter means the difference between organs; lowercase letter means difference between solvents (p ≤ 0.05).

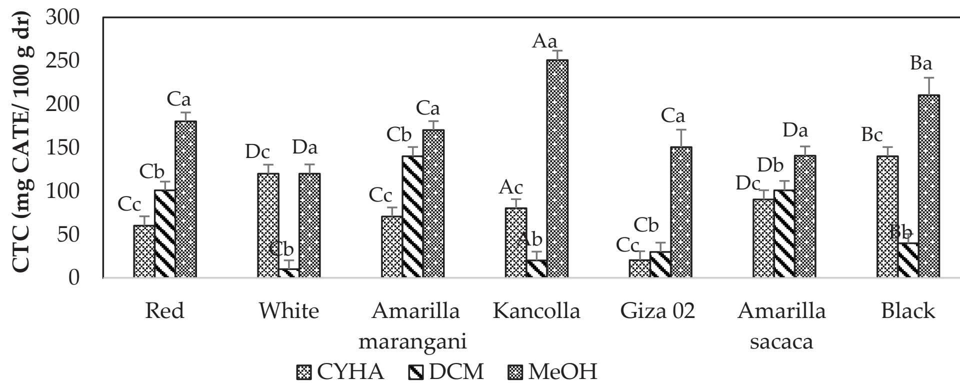

Figure 3. Condensed tannin content in the different extracts of quinoa genotypes. CYHA: cyclohexane; DCM: dichloromethane; MeOH: methanol. Means' values ± SD (n=3); different letters on the histograms mean a significant difference (P ≤ 0.05).

MeOH-Giza 02 showed the highest TFC (490.1 mg QE/100 g dr), followed by DCM-*A. marangani* (300.9 mg QE/100 g dr), and finally CYHA-black (220.8 mg QE/100 g dr). Statistically, there was a significant difference between the species and between and the extraction solvents (p > 0.05). The current results were higher than those found by Park *et al.* (2017), when working on ethanolic extracts of quinoa seeds cultivated in Korea, the USA, and Peru. They found, respectively, an amount of 20.9, 13.24, and 11.5 mg QE/100 g dr.

CTC was significantly different depending on both the extracts of the quino genotypes and the extracting solvents (Figure 3). The highest values were detected in Kancolla and Black extracted with MeOH, whereas the lowest value was found in DCM-White extract.

Determination of RSC

The RSC was quantified using the DNSA technique. As shown in Table 3, the contents of reducing sugars in different quinoa seed extracts varied depending on the polarity of the used solvents (successively). Notably, as expected, the CYHA and DCM extracts showed low RSC not exceeding 78.4 and 183.1 mg GE/100 g dr, respectively (Table 3). However, the MeOH extracts exhibited high sugar contents ranging from 425.4 mg GE/100 g dr for *A. marangani* to 1202.1 mg GE/100 g dr, for *A. sacaca.* These findings highlight the richness of quinoa seeds in sugars and the influence of the solvent used in their extraction.

Table 3. Reducing sugar content in the different extracts of quinoa genotypes.

	Reducing sugar content (mg GE/ g dr)		
Species/solvents	**CYHA**	**DCM**	**MeOH**
Red	24.3±1.1^{d}	7.6±0.8^{d}	727.4±65.5^{d}
White	18.0±2.5^{e}	31.1±1.2^{c}	979.3±7.5^{a}
Amarilla marangani	24.1±0.7^{d}	35.5±2.3^{c}	425.4±3.7^{f}
Kancolla	78.4±4.9^{a}	52.7±4.6^{b}	698.6±125.0^{d}
Giza 02	53.9±4.0^{b}	183.1±30.4^{a}	895.8±3.8^{c}
Amarilla sacaca	40.3±4.7^{c}	51.9±3.0^{b}	907.9±4.1^{b}
Black	52.6±3.0^{b}	52.7±4.3^{b}	536.9±22.3^{e}

CYHA: cyclohexane; DCM: dichloromethane; MeOH: methanol. Values with different superscript letters (a, b, and c), in the same column, are significantly different at p < 0.05.

The current results showed that MeOH solvent was more efficient in extracting phytochemicals than other solvents. Based on data from the existing literature, the reduced sugar content of quinoa seeds surpasses that of the aqueous extract from Titicaca, Viking, and puno quinoa seeds, which accounted, respectively, for only 43.4, 49.6, and 47.5% of the total extract weight (Vităneașcu *et al.*, 2019).

HPLC analysis

The phenolic compounds of the different extracts of quinoa genotypes were identified using the HPLC-DAD analysis. For each chromatogram, based on the comparison of the retention time and DAD spectra of the standard compound analyzed under the same conditions and the comparison with the literature, the phenolic compounds were identified. In total, seven phenolic compounds were identified and quantified in MeOH extracts in the different quinoa genotypes (Table 4).

In general, variations exist across quinoa genotypes concerning the levels of phenolic chemicals present. The seven phenolic compounds were identified and categorized into four types: phenolic acids, which consist of four compounds (gallic acid, caffeic acid, coumaric acid, and hydroxycinnamic acid), flavonoids (catechin hydrate), stilbenes (polydatin), and coumarins

Table 4. HPLC-DAD analysis and concentrations of compounds, tentatively identified in quinoa seeds.

			Concentration (mg/g dr)						
No.	**Compounds**	**RT (min)**	**Red**	**White**	**A. marangani**	**Kancolla**	**Giza 02**	**A. sacaca**	**Black**
1	Gallic acid	7.96	0.1	0.3	nd	10.6	nd	0.2	0.3
2	Polydatin	8.21	nd	0.2	nd	nd	nd	nd	0.2
3	Catechin hydrate	16.53	nd	nd	nd	0.4	nd	0.2	nd
4	Coumaric acid	24.67	0.1	nd	0.6	nd	nd	nd	nd
5	Hydorxycinnamic acid	25.58	0.5	nd	nd	0.13	nd	0.1	nd
6	Caffeic acid	26.45	nd	6.4	nd	3.8	3.9	nd	6.8
7	7-Hydroxycoumarin	26.65	0.4	nd	0.1	nd	0.5	nd	nd

RT: retention time; nd: not detected.

(7-hydroxycoumarin). Nevertheless, the presence of gallic acid was observed in five samples, each with varying quantities ranging from 0.1 mg/g dr for red to 10.6 mg/g dr for kancolla (Table 4).

This chemical had a higher prevalence compared to the other compounds that were present. The findings of this study are consistent with the research conducted by Melini F. and Melini V. (2022), which revealed that gallic acid had the most significant concentrations compared to other compounds identified in Chinese quinoa. Furthermore, caffeic acid was observed in white, Giza 02, and black quinoa, with concentrations of 6.4, 3.9, and 6.8, respectively. This chemical was identified in quinoa extract (80% acetone) at concentrations ranging from 2.17 to 3.81 mg/g. Specific quinoa components had lower concentration levels. The observed concentrations of the identified compounds are limited to a maximum of 0.5 mg/g for red, 0.6 mg/g for *A. marangani*, and 0.2 mg/g for *A. sacaca*. Certain chemicals were previously undetectable in quinoa preparations. The substances that have been recently identified include polydatin and 7-hydroxycoumarin. The first molecule was identified in white (0.2 mg/g) and black (0.2 mg/g) quinoa, but the subsequent molecule was seen in red (0.4 mg/g), *A. marangani* (0.1 mg/g), and Giza 02 (0.5mg/g) quinoa.

Biological activities

Antioxidant activity of quinoa seeds extracts

Table 5 showed the results of antioxidant activity, of the quinoa seeds extracts, evaluated by DPPH methods. Statistically, there was a significant difference (p≤ 0.05) between the different solvents, as well as between some quinoa genotypes, in terms of antioxidant activity. All the species extracts highlighted an inhibition percentage which exceeded 50%; thus, the transition to the calculation of the minimum concentration that inhibits 50% of the radicals (IC_{50}) was essential. The lower the IC_{50} value, the higher the antioxidant activity. Nevertheless, the varied IC_{50} values ranged between 38.5 µg/mL, for CYHA-*A. marangani* extract, and 6.4 µg/mL with MeOH extract of three quinoa genotypes (white, kancolla, and Giza 02).

Table 5. Antioxidant activity of organic extracts from different quinoa genotypes. IC_{50} value (µg/mL).

	IC_{50} (µg/mL)		
Species/solvents	**CYHA**	**DCM**	**MeOH**
Red	18.0 ± 1.6^{c}	11.0 ± 1.1^{c}	6.8 ± 1.1^{a}
White	18.5 ± 2.9^{c}	37.0 ± 3.4^{a}	6.4 ± 1.2^{b}
Amarilla marangani	38.5 ± 3.6^{a}	13.0 ± 1.3^{c}	7.2 ± 0.9^{a}
Kancolla	15.5 ± 2.1^{d}	29.0 ± 2.0^{b}	6.4 ± 1.8^{b}
Giza 02	18.0 ± 1.9^{c}	26.5 ± 2.1^{b}	6.4 ± 1.7^{b}
Amarilla sacaca	13.5 ± 1.4^{d}	35.5 ± 3.4^{a}	7.5 ± 1.2^{a}
Black	22.5 ± 2.3^{b}	34.0 ± 3.5^{a}	7.7 ± 1.7^{a}

CYHA: cyclohexane; DCM: dichloromethane; MeOH: methanol. Values with different superscript letters (a, b, and c), in the same column, are significantly different at $p < 0.05$.

The previous study of Liu M *et al.* (2020) was in agreement with our study. They also found that the red quinoa was higher than black quinoa. Some reports suggested a good correlation between TFC/CTC extracted from quinoa varieties (red, black, or white) and DPPH inhibition activity (Ocampo *et al.,* 2023). Those findings were confirmed in this study by a good significant correlation between TFC/DPPH (r = 0.8) and CTC/DPPH (r = 0.7). In addition, there was a moderate correlation found between TPC/DPPH and RSC/ DPPH with an r-value of 0.63, and 0.65, respectively (Table 7).

Antibacterial activity

Table 6 presents the measurements of the growth inhibition zone width for several quinoa seed extracts against gram-positive bacteria (*L. monocytogenes* and *M. luteus*) and gram-negative bacteria (*E. coli* and *S. typhimurium*).

Table 6. Antimicrobial activity of different quinoa genotypes extracts against bacterial strains.

Genotypes		E. coli	S. typhimurium	M. luteus	L. monocytogenes
Diameter of inhibition zone (mm)					
Red	*CYHA*	6±1.3[b]	5±1.2[b]	11±1.7[b]	2±0.2[c]
	DCM	4±0.1[b]	2±0.3[c]	6±1.1[c]	1±0.1[c]
	MeOH	3±0.1[c]	4±0.9[b]	4±0.6[c]	2±0.4[c]
White	*CYHA*	1±0.1[d]	1±0.2[d]	1±0.1[d]	1±0.1[c]
	DCM	1±0.3[d]	1±0.3[d]	2±0.5[d]	1±0.1[c]
	MeOH	1±0.4[d]	2±0.3[c]	2±0.2[d]	1±0.1[c]
Amarilla marangani	*CYHA*	1±0.2[d]	1±0.1[d]	1±0.1[d]	1±0.1[c]
	DCM	1±0.3[d]	1±0.1[d]	1±0.1[d]	1±0.1[c]
	MeOH	1±0.1[d]	2±0.3[c]	1±0.2[d]	2±0.2[c]
Kancolla	*CYHA*	na	4±0.4[b]	2±0.3[d]	2±0.4[c]
	DCM	na	Na	1±0.1[d]	Na
	MeOH	na	Na	1±0.1[d]	Na
Giza 02	*CYHA*	2±0.2[d]	1±0.1[d]	6±0.4[c]	1±0.1[c]
	DCM	1±0.1[d]	Na	3±0.2[d]	1±0.1[c]
	MeOH	2±0.3[d]	1±0.1[d]	5±0.3[c]	1±0.1[c]
Amarilla sacaca	*CYHA*	Na	Na	Na	3±0.5[b]
	DCM	Na	Na	3±0.4[d]	1±0.1[c]
	MeOH	Na	Na	Na	Na
Black	*CYHA*	4±0.2[b]	2±0.7[c]	6±0.8[c]	1±0.1[c]
	DCM	1±0.1[d]	1±0.1[d]	1±0.1[d]	1±0.1[c]
	MeOH	1±0.1[d]	1±0.3[d]	1±0.2[d]	1±0.1[c]
Ampicillin		18.4±0.8[a]	14.5±0.4[a]	17.6±1.1[a]	11±0.2[a]

Na: not active; Values with different superscript letters (a, b, and c), in the same column, are significantly different at $p < 0.05$.

Table 7. Pearson's correlation matrix.

Variables	TPC	TFC	CTC	DPPH	RSC	E. coli	L. monocytogenes	M. luteus	S. typhimurium
TPC	1	0.68	0.56	−0.63	0.40	0.34	−0.09	0.08	0
TFC	0.68	1	0.89	−0.8	0.73	0.03	0.01	−0.13	0.08
CTC	0.56	0.89	1	−0.71	0.58	−0.04	−0.07	−0.18	0.03
DPPH	−0.63	−0.79	−0.70	1	−0.65	−0.11	−0.14	−0.04	−0.24
RSC	0.40	0.73	0.58	−0.65	1	−0.15	−0.26	−0.15	−0.06
E. coli	0.34	0.03	−0.04	−0.11	−0.15	1	0.25	0.90	0.67
L. monocytogenes	−0.09	0.01	−0.07	−0.14	−0.26	0.25	1	0.19	0.52
M. luteus	0.08	−0.13	−0.18	−0.04	−0.15	0.90	0.19	1	0.60
S. typhimurium	0	0.08	0.03	−0.24	−0.06	0.67	0.52	0.60	1

The antibacterial activity of the different extracts was assessed using the agar well diffusion technique (Table 6). The table indicates that the various extracts exhibit varying levels of activity, ranging from low to moderate or nil, against the other microorganisms. Red quinoa had the most significant inhibitory diameter against both gram-positive and gram-negative bacteria among all the extracts. The inhibitory diameter of CYHA-red quinoa was observed to be 6, 5, and 11 mm against *E.coli*, *S. typhimurium*, and *M. luteus*, respectively. Nevertheless, *L. monocytogenes* exhibited the highest resistance to several extracts, including CYH-red. The findings were moderately aligned with the results reported in the study conducted by Rahimi and Bagheri (2020). The researchers observed that the ethanolic extract derived from *Chenopodium quinoa* red carina exhibited a diameter of inhibition, around 10 mm, against *E. coli* bacteria. Furthermore, the CYHA extract derived from Giza 02 and black quinoa exhibited a modest amount of inhibition, measuring 6 and 5 mm, respectively, against

M. luteus. The current findings exhibited a minor proximity to the results reported by Park *et al.* (2017). The researchers observed that *L. monocytogenes* exhibited resistance to the quinoa extract, but *E. coli* and *S. typhimurium* showed susceptibility to the same extract. The inhibition zones of these two bacteria were measured to be 7.04 and 7.06, respectively.

The inhibition zone of the remaining quinoa extracts was found to be less than 4 mm. The plant solvent extracts exhibit minor sensitivity toward microorganisms, as shown in Table 6. It is well recognized that quinoa species have a high concentration of phenolic chemicals, including flavonoids, phenolic acids, and saponin compounds (Pathan and Siddiqui, 2022). Statistical analysis revealed a weak or inverse association between TPC/TFC/CTC and the various bacterial strains, as seen in Table 7. Hence, there is potential for the different phenolic compounds to have limited efficacy against the remaining bacterial strains. The observed absence of action in quinoa extracts may be attributed to many chemicals' potential synergistic or additive impact.

Multivariate analysis

A PCA was conducted in order to get an insight into the interrelationship among the variables that were assessed. Figure 4 displays the results of PCA, whereby the primary components (F1 and F2) account for 71.30% of the overall variation in the data. The retention of axes was justified due to their expression of 41.01% (F1) and 30.28% (F2). Concurrently, the PCA loading plot indicated strong connections between the principal components and the original variables, as well as between the various activities and the phytochemical composition (TPC, TFC, or CTC).

Figure 4 showed a strong positive association between the F1 axis and TFC and CTC, with loadings of 0.97 and

Table 8. Correlation between factors and variables.

	F1	F2	F3
TPC	0.76	0.14	−0.32
TFC	0.96	−0.03	0.10
CTC	0.88	−0.10	0.10
DPPH	−0.89	−0.15	−0.16
RSC	0.78	−0.22	−0.08
E. coli	0.08	0.92	−0.34
L. monocytogenes	−0.06	0.53	0.77
M. luteus	−0.07	0.88	−0.35
S. typhimurium	0.09	0.85	0.27

0.88, respectively (Table 8). Furthermore, the MeOH-Giza 02 extract exhibited higher proximity to the specified criteria. In addition, the F1 axis had a strong association, albeit it contained only one parameter on the opposing side. There was a strong negative correlation (r = −0.89) seen between F1 and DPPH. Based on the observed distribution on F1, it can be inferred that TPC exhibited a symmetrical relationship with the antioxidant activity, as shown by the IC_{50} value. This implies that the chemicals significantly increased the inhibition of the said activity. Furthermore, it was observed that the second axis F2 strongly correlated with the three bacterial strains, namely, *E. coli*, *M. luteus*, and *S. Typhimurium*. This correlation was assessed using r-values of 0.92, 0.88, and 0.85, respectively, as shown in Table 8. In contrast, the fourth bacterial strain, *L. monocytogenes*, had a strong correlation with the F3 axis, as shown by a correlation coefficient of r = 0.77 (Table 8).

Further, the apparent relationship between the different quinoa genotypes extracts was expressed using a hierarchical clustering dendrogram based on the Euclidean distance (Figure 6).

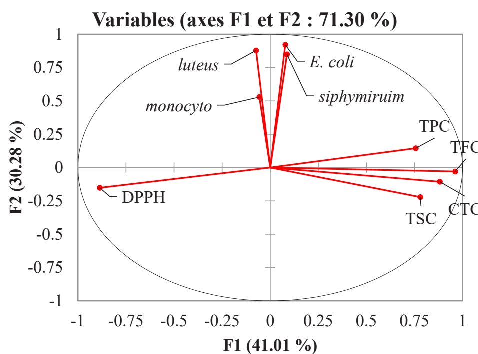

Figure 4. Principal components analysis loading plot of antimicrobial activities of *C. quinoa* seeds extracts.

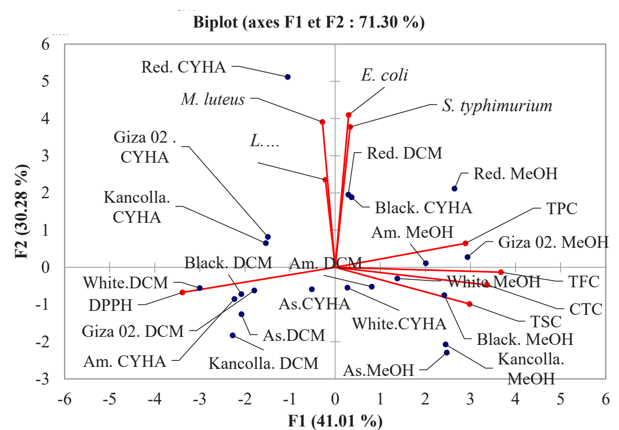

Figure 5. Principal components analysis biplot of biological activities of different quinoa genotypes extracts.

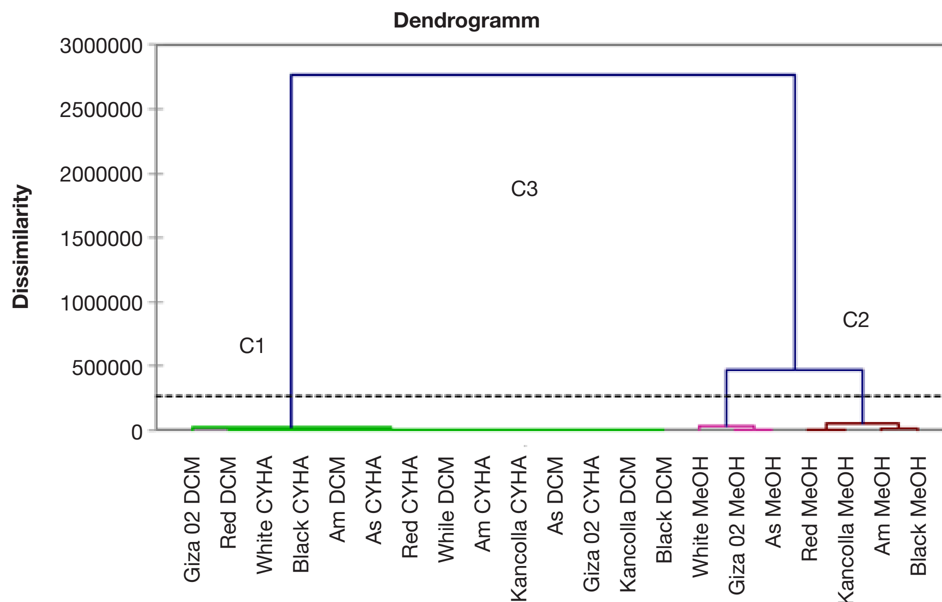

Figure 6. Hierarchical clustering dendrogram with clustering distance of different quinoa genotypes extracts. The X-axis represents the different quinoa genotypes extracts, and the Y-axis represents the clustering distance.

The hierarchical clustering dendrogram was also used to depict the apparent association among the various quinoa genotype extracts, using the Euclidean distance as the basis (Figure 6). Hierarchical clustering is a method used to display data in a dendrogram, which is a cluster tree. In this approach, each grouping is connected to two or more points based on their clustering distance (Karna *et al.,* 2022). Based on the biochemical characteristics and biological potentialities, the dendrogram findings successfully categorized the extracts from several quinoa genotypes into distinct clusters. The findings revealed a strong correlation between the methanolic extracts of white, Giza 02, and *A. sacaca*, as they formed a cluster with a low clustering distance across all samples. Based on the data shown in Table 9, the mentioned extracts were categorized based on their significant levels of TPC, TFC, and RSC, as well as their notable antioxidant capacity. In addition, it was observed that the methanolic extracts of the other quinoa genotypes (red, kancolla, *A. marangani*, and black) exhibited a strong correlation and formed a cohesive clustering group. This was categorized based on their elevated CTC (Table 9).

All the DCM and CYHA extracts of quinoa genotypes expressed a close relationship and formed a clustering group characterized by their low amounts of phytochemicals and potency against DPPH radical and the bacterial strains, except *M. luteus*.

Conclusions

The nutritional research revealed that the seven quinoa genotypes had similar values in each parameter, including protein, fat, ash, and carbohydrates. The MeOH extracts had the most significant levels of phenolic content and RSC across all quinoa genotypes, as seen in the phytochemical study. Furthermore, the extract above exhibited considerable antioxidant activity, likely attributed to the various phenolic components detected by HPLC-DAD analysis. Nevertheless, the antibacterial activity exhibited selectivity among the different types of extracts. The CYHA extract from red quinoa had the highest efficacy against several bactrian strains, except *L. monocytogenes*. Collectively, our findings substantiate the potential of quinoa as a pseudo-cereal and its use as a prominent food crop on a global scale.

Table 9. Central objects of the hierarchical clustering dendrogram.

Class	TPC	TFC	CTC	DPPH	RSC	E. coli	L. monocytogenes	M. luteus	S. typhimunium
1 (Giza 02. CYHA)	46.78	9.70	2.60	18.00	53.86	2.00	1.00	6.00	1.00
2 (Black. MeOH)	63.44	43.84	21.46	7.70	536.92	1.00	1.00	1.00	1.00
3 (Giza 02. MeOH)	66.41	49.06	15.72	6.40	1170.42	2.00	1.00	5.00	1.00

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

The authors declare no conflict of interest.

Authors' contributions

All authors have read and agreed to the published version of the manuscript.

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