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Chenopodium quinoa Willd. (quinoa) grains: A good source of phenolic compounds

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ABSTRACT

The ingestion of bioactive compounds has revealed health benefits, namely in the prevention and/or treatment of several diseases. This work aims to characterize the phenolic profile of three colour varieties of *Chenopodium quinoa* Willd. grains (black, red and white), and also evaluate their cytotoxic and antimicrobial activity. All varieties revealed the presence of phenolic compounds, namely, quercetin and kaempferol derivatives. In this study, quinoa grains did not reveal any anti-proliferative capacity in tumour cell lines, and, as expected, they were devoid of any toxicity. All of the analysed extracts possessed antibacterial and antifungal activities (inhibitory and bactericidal/fungicidal) against the microbial strains considered, exhibiting promising values of minimum bactericidal concentrations (mean MBC 0.153–0.916 mg/mL) and minimum fungicidal concentrations (mean MFC 0.211–0.884 mg/mL). Quinoa varieties represent a good source of bioactive compounds, interfering beneficially in the organism, specifically as antimicrobial agents. Thus, these extracts could be used in the development of bioactive ingredients.

1. Introduction

The contemporary lifestyle is presented as one of the principal causes for the abundant emergence of chronic diseases, having become a very common problem in modern society (Balch, 2006). In order to combat this problem, the consumer has opted for changing their eating habits, and choosing foods that, besides being a good source of nutrients, are also rich in compounds with health benefits (Betoret, Betoret, Vidal, & Fito, 2011; Barbé et al., 2014). In this way, the search for foods with vitamins, carotenoids and polyphenols in their composition is increasing (Küster & Vila, 2017; Recharla, Riaz, Ko, & Park, 2017).

Phenolic compounds possess a great importance in the organism, intervening beneficially in the prevention or treatment of numerous health related issues, namely, diabetes, cancer, cardiovascular problems, infections, obesity, neurodegenerative diseases and others (Esfanjani, Assadpour, & Jafari, 2018; Qin, Wang, Liu, Zhang, Li, & Wu, 2018). All this therapeutic capacity is due to the cardioprotective, vasodilating, anti-tumour, anti-inflammatory, anti-oxidative, antimicrobial, anti-hypertensive and antihyperlipidemic action exerted by these compounds (Croft, 2016; Croft et al., 2018). Hence, phenolic compounds are one of the most studied types of compounds, not only in the evaluation of their activities in *in vitro* assays, but also in assays of bioavailability in metabolism after ingestion (Castello et al., 2018; Croft, 2016).

Chenopodium quinoa Willd. belongs to the Amaranthaceae family and its grains are nowadays cultivated in different parts of the world (Multari, Marsol-Vall, Keskitalo, Yang, & Suomela, 2018). Recently, the production and consumption of this pseudocereal has increased due to the great nutritional value and the presence of bioactive compounds (Li & Zhu, 2018; Multari, Marsol-Vall, Keskitalo, Yang, & Suomela, 2018). Besides playing an important role in combating malnutrition in developing countries, quinoa is also considered an ideal food for all consumers, principally for vegetarians, health-conscious people, athletes, the elderly and people with celiac disease, because it is gluten-free (Agza, Bekele, & Shiferaw, 2018; Li & Zhu, 2018). Beyond the bioactive characteristics, these seeds also present conservation benefits, since its low moisture content prolongs its durability, creating a hostile environment for the microorganisms and preventing chemical and enzymatic reactions that potentiate the product's deterioration (Multari, Marsol-Vall, Keskitalo, Yang, & Suomela, 2018). Several studies have focused on these grains; however, it is necessary to assess more

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parameters that characterize the edible seeds of *Chenopodium quinoa* Willd, in order to better explore other options for use and application in the industry, with a view towards exploiting the potential benefit to the consumer's health. In this way, the novelty of this research work is the detailed study of the phenolic profile of quinoa grains belonging to three different colour varieties, and the evaluation of their cytotoxic and antimicrobial (antibacterial and antifungal) activity.

2. Materials and methods

2.1. Samples

Samples from the three colour varieties (black, red and white) of *Chenopodium quinoa* Willd. saponified by an unknown process, were provided by the National Agricultural University La Molina (Peru) (10 samples), others acquired in a commercial establishment of Peru (27 samples), and a few samples were obtained from commercial establishments in Spain (2 samples), according a table previously published by (Pereira et al., 2019). In this study 39 samples were used, being 11 samples belong to the red variety, 9 samples belong to the black variety and 19 samples belong to the white variety. The grains were ground to a fine dry powder ~ 20 g mesh and homogenized.

2.2. Determination of phenolic profile

2.2.1. Extraction procedure

Samples from the three varieties of quinoa grains was powdered, and the homogeneous mixture was extracted separately, using a hydroethanolic solution (ethanol/water; 80:20, *v/v*) as solvent. The samples (approximately 1 g) were extracted by macerating with 30 ml of hydroethanolic solution (25 °C at 150 rpm) for 1 h. After the maceration process, the supernatant was filtered using filter paper (Whatman No. 4) and the residue was re-extracted by adding again, 30 ml of the same solvent mixture. The obtained extracts were evaporated at 40 °C (rotary evaporator Büchi R-210, Flawil, Switzerland) in order to remove the ethanolic fraction, and then the aqueous fraction was frozen and lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA). The lyophilized extract (10 mg) was redissolved in 2 ml of ethanol/water in order to obtain a final concentration of 5 mg/mL, for later analysis in a HPLC-DAD-ESI/MS (Lopes et al., 2018).

2.2.2. Determination of phenolic profile

A solution of 5 mg/mL was made, using the lyophilized extract and ethanol/water solution (20:80, *v/v*). A procedure described by Bessada, Barreira, Barros, Ferreira, & Oliveira (2016) was applied for chromatographic evaluation. Detection was performed using a LC-DAD-ESI/MSn system (Dionex Ultimate 3000 UPLC; Thermo Scientific, San Jose, CA, USA) equipped with a diode array detector (DAD using 280, 330, and 370 nm as wavelengths) and an electrospray ionization mass detector (Linear Ion Trap LTQ XL, ThermoFinnigan, San Jose, CA, USA) working in negative mode. Identification of compounds were undertaken by comparing several chromatographic parameters with those available in literature and with the commercial standards (apigenin-6-*C*-glucoside, caffeic acid, chlorogenic acid, hesperetin, luteolin-7-*O*-glucoside, naringenin, quercetin-3-*O*-rutinoside, rosmarinic acid). Calibration curves were obtained through the DAD by using the previously mentioned standards for quantification, and the results were expressed as mg/g of extract.

2.3. Evaluation of the bioactivity of hydroethanolic extract obtained from quinoa grains

2.3.1. Cytotoxicity evaluation

The cytotoxicity assay was performed employing the methodology described in Barros et al. (2013). Four tumour cell lines: HeLa (cervical carcinoma), HepG2 (hepatocellular carcinoma), MCF-7 (breast adenocarcinoma) and NCI-H460 (non-small cell lung cancer), and a primary cell culture derived from pig liver (PLP2) was tested in the anti-proliferative capacity of cells and hepatotoxicity, respectively. These cell lines were maintained as adherent cultures in RPMI-1640 medium supplemented with 10% FBS (MCF-7 and NCI-H460) or in DMEM supplemented with 10% FBS, 2 mM glutamine, 100 U/mL penicillin and 100 mg/mL streptomycin (HeLa and HepG2 cells), at 37 °C in an incubator with humidified air and 5% CO_2. A positive control (Ellipticine) was used and the results were expressed in GI_{50} values (sample concentration that inhibits the growth of cells by 50%) given in μg/mL.

2.3.2. Determination of antimicrobial activity – Microdilution assay

The methodology proposed by Sokovic, Glamoclija, Marin, Brkic, & van Griensven (2010) and Sokovic & van Griensven (2006) was utilized in the determination of minimum inhibitory concentration (MIC) for bacteria and fungi, minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC).

The lyophilized extract was diluted with water at a concentration of 10 mg/mL. For the antibacterial evaluation, several strains of Gram-positive (*Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (clinical isolate) and *Listeria monocytogenes* (NCTC7973), and Gram-negative *Escherichia coli* (ATCC 35210), *Salmonella* Typhimurium (ATCC 13311), and *Enterobacter cloacae* (ATCC 35030)) bacteria were assayed. Also, for the antifungal evaluation, several microfungi strains (*Aspergillus fumigatus* (ATCC1022), *Aspergillus ochraceus* (ATCC12066), *Aspergillus niger* (ATCC6275), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC9112) and *Penicillium verrucosum* var. *cyclopium* (food isolate)) were used. The positive controls chosen for the antibacterial evaluation were streptomycin and ampicillin, while those for the antifungal analysis were ketoconazole and bifonazole. The results were expressed in mg/mL. All of the assays were performed in triplicate.

2.4. Statistical analysis

For each of the quinoa bioactive properties, the effect of quinoa colour varieties was assessed by adjusting the linear model,

$$y_{ij} = \mu + \alpha_i + \epsilon_{ij}$$

where y_{ij} is the observed value for the i-th quinoa sample of the j-th quinoa colour, μ is the overall mean value, α_i is the main effect of the i-th quinoa colour, and ϵ_{ij} is the random error attributed to the observation y_{ij}. The linear models were fitted using the "lm()" function, while the analysis of variance of every model was produced with the "anova" function. The "emmeans" library was used to compute the least-square means (LSM) and LSM-pairwise comparisons between quinoa colour varieties using the Tukey's Honestly Significant Difference test. The homogeneity of variance was assessed by the Levenés test, and the residuals normality was evaluated by the Shapiro Wilk test from the "car" package. All of the libraries and functions aforementioned were implemented in the R software (version 3.4.4, R CoreTeam, Austria).

3. Results and discussion

3.1. Phenolic profile evaluated in hydroethanolic extract of quinoa grains

The detailed phenolic composition of quinoa grains (black, red and white varieties) are presented in Table 1 and the phenolic profile can be visualized in Fig. 1. The extraction technique applied in this scientific study is a conventional method widely used in the extraction of bioactive compounds and optimized by our working group. It is a simple technique, more economically accessible, easier to apply and environmentally friendly, not causing the emission of greenhouse gases that affect the environmental impact. In addition, organic solvents were used depending on the polarity, with no depletion of the plant raw material, either by saturation of the extraction solvent or by the use of a

Table 1
Retention time (Rt), wavelengths of maximum absorption in the visible region (λmax), mass spectral data, identification and tentative quantification of phenolic compounds in hydroethanolic extracts obtained from *C. quinoa* grains of black, red and white varieties. The results are present as mean ± SE.

Peak	Rt (min)	λ_{max} (nm)	$[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	Tentative identification	Black (μg/mL)	Red (μg/mL)	White (μg/mL)
1	14,00	352	755	760(38),301(100)	Quercetin-3-*O*-(2″,6″-di-*O*-α-l-rhamnoside)-β-d-galactoside	189.4 ± 18.9^{a}	127.8 ± 17.1^{b}	79.8 ± 13.0^{c}
2	15,16	353	741	609(100),301(80)	Quercetin-3-*O*-(2″-*O*-β-apioside-6″-*O*-α-rhamnoside)-β-galactoside	182.1 ± 13.16^{a}	41.7 ± 11.90^{b}	41.2 ± 9.05^{b}
3	15,79	265,348	739	593(44),285(100)	Kaempferol-3-*O*-(2″,6″-di-*O*-α-rhamnoside)-β-glucoside	148.8 ± 30.6^{b}	62.7 ± 38.7^{b}	282.1 ± 19.9^{a}
4	16,32	353	609	301(100)	Quercetin-3-*O*-rutinoside	31.5 ± 13.8^{b}	82.7 ± 11.0^{a}	11.5 ± 15.0^{b}
5	16,52	255,353	595	463(33),301(100)	Quercetin-3-*O*-[β-D-apiofuranosyl(1‴→2″)]- β -D galactopyranoside	13.2 ± 3.96^{b}	31.3 ± 3.16^{a}	9.6 ± 2.54^{b}
6	17,15	248,328	725	593(66),285(100)	Kaempferol-3-*O*-[β-D-apiofuranosyl(1″→2′)- α-L-rhamnopyranosyl(1‴→6′)]-β-D-galactopyranoside	22.1 ± 7.27^{b}	16.7 ± 6.58^{b}	48.0 ± 5.01^{a}
7	17,42	254,345	477	301(100)	Quercetin-*O*-glucuronide	11.5 ± 3.96^{b}	24.2 ± 3.38^{a}	7.0 ± 2.64^{b}
8	18,80	265,347	593	285(100)	Kaempferol-3-*O*-rutinoside	3.84 ± 1.67^{b}	5.62 ± 1.51^{b}	13.47 ± 1.15^{a}
					TPC	**574.7 ± 101.64^{a}**	**358.7 ± 88.21^{b}**	**483.4 ± 82^{a}**

SE – standard error; TPC – total phenolic compounds; Standard calibration curves: quercetin-3-*O*-glucoside (y = 34843x − 160173, R^2 = 0.9998; LOD 0.21 μg/mL; LOQ 0.71 μg/mL); kaempferol-3-*O*-glucoside (y = 11117x + 30861, R^2 = 0.9999; LOD 0.15 μg/mL; LOQ 0.41 μg/mL). In each row different letters mean significant differences among total compounds ($p < 0.10$).

diffusional liquid between the extraction medium and the interior of the vegetable cells. In general, the evaluated extracts revealed the presence of eight phenolic compounds, namely quercetin and kaempferol glycoside derivatives. The identification of these compounds was performed taking into account the retention time, UV–Vis spectra, mass fragmentation pattern, commercial standards and information present in literature.

The compounds belonging to the peaks 1, 2, 4, 5, and 7 were tentatively identified as quercetin glycoside derivatives, namely, quercetin-3-*O*-(2″,6″-di-*O*-α-l-rhamnoside)-β-d-galactoside, quercetin-3-*O*-(2″-O-β-apioside-6″-*O*-α-rhamnoside)-β-galactoside, quercetin-3-*O*-rutinoside, quercetin-3-*O*-[β-D-apiofuranosyl(1‴→2″)]-β-D galactopyranoside and quercetin-*O*-glucuronide, respectively. These identifications were made according to previous studies conducted by Hirose, Fujita, Ishii, & Ueno (2010) and Gómez-Caravaca, Segura-Carretero, Fernández-Gutiérrez, & Caboni (2011). Quercetin is present in large quantities in many fruits and vegetables. According to several preclinical and clinical studies, this compound has exhibited some biological activities, namely: anti-inflammatory, antidiabetic, hepatoprotective, neuroprotective, antiplatelet, antibacterial potential, and acts to eliminate free radicals (Khursheed, Singh, Wadhwa, Gulati, & Awasthia 2019).

The compounds belonging to the peaks 3, 6, and 8 were tentatively identified as kampferol glycoside derivatives, namely kaempferol-3-*O*-(2″,6″-di-*O*-α-rhamnoside)-β-glucoside,kaempferol-3-*O*-[β-D-apiofuranosyl(1″→2′)-α-L-rhamnopyranosyl(1‴→6′)]-β-D-galactopyranoside and kaempferol-3-*O*-rutinoside, respectively. These peaks have also been identified and described previously by Hirose, Fujita, Ishii, & Ueno (2010) and Gómez-Caravaca, Segura-Carretero, Fernández-Gutiérrez, & Caboni (2011). Kaempferol is widely found in plant matrices and traditional medicines. According to previous studies, this compound has shown beneficial biological functions, mainly antioxidant and anti-inflammatory effects, reported in cultured microglia and inflammatory animal models (Li et al., 2019).

The quinoa colour variety was found to significantly affect the concentration of each compound of the extracts. Compounds 1 and 2 were greater in the black variety, with concentration values of 189.4 ± 18.9 and 182.1 ± 13.16 μg/mL, respectively, whereas peaks 3 (282.1 ± 19.9 μg/mL), 6 (48.0 ± 5.01 μg/mL) and 8 (13.47 ± 1.15 μg/mL) showed higher contents in the white variety. Extracts from the red quinoa variety exhibited higher concentrations for the molecules identified in the peaks 4 (82.7 ± 11.0 μg/mL), 5 (31.3 ± 3.16 μg/mL) and 7 (24.2 ± 3.38 μg/mL). Such heterogeneity observed in the three colour varieties of quinoa grains did not take place only in the individual profile of phenolic compounds, but also in other chemical and nutritional parameters (Pereira et al., 2019). This discrepancy has been recently described by Aguilar, Miano, Obregón, Soriano-Colchado, & Barraza-Jáuregui (2019) and Vera, Alca, Saravia, Campioni, & Alpuy (2019) when studying different cultivars of quinoa. In this sense, it is important to note that the disparity in chemical and nutritional composition between samples of the same species may depend not only on geographic factors or maturity, but also on the different varieties, cultivars and accessions to which they belong.

A few authors have studied the phenolic composition of quinoa species. For instance, Gómez-Caravaca, Iafelice, Verardo, Marconi, & Caboni (2014) studied the influence of the pearling process on the phenolic content in quinoa. The extraction was made in an ultrasonic bat using a solution of methanol/water (4:1, *v/v*) with 0.1% of acetic acid as solvent. The evaluation of phenolic profile was made through high performance liquid chromatography coupled to DAD and mass spectrometer detectors (HPLC–DAD–ESI-MS), and the results showed the presence of flavonoids and phenolic acids, namely, protocatechuic acid, ferulic acid, quercetin and kaempferol derivatives, rutin and others. The total content of phenolic compounds was 261.04 ± 3.68 mg/100 g sample. In a more recent study, Pellegrini et al. (2018) evaluated the polyphenols present in different varieties of black, red and white quinoa seeds from local markets in Spain, Bolivia and Peru. The extraction procedure was made with two consecutive

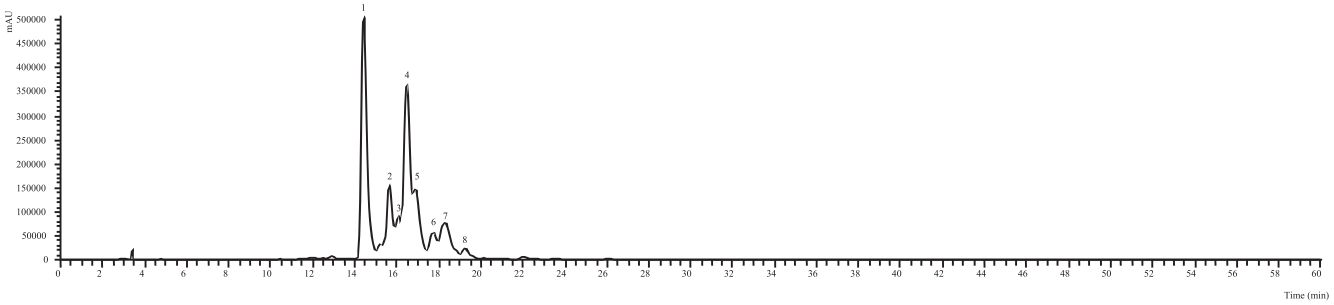

Fig. 1. Chromatographic profile of a sample of *C. quinoa* grains belonging to a red variety, at 280 nm. The peak numbers correspond to the compounds listed in Table 1.

Table 2
Cytotoxic activity (in tumour and non-tumour cell lines, GI_{50}, μg/mL) of *C. quinoa* Willd. grains hydroethanolic extract. The results are present as mean ± SE.

	HeLa	HepG2	MCF-7	NCI-H460	PLP2
Black	> 400	> 400	> 400	> 400	> 400
Red	> 400	> 400	> 400	> 400	> 400
White	> 400	> 400	> 400	> 400	> 400
Ellipticine	1.91 ± 0.06	1.1 ± 0.2	0.91 ± 0.04	1.0 ± 0.1	3.2 ± 0.7

SE - standard error; HeLa: cervical carcinoma; HepG2: hepatocellular carcinoma; MCF-7: breast carcinoma; NCI-H460: nan-small cell lung cancer; PLP2: non-tumour primary cells culture. GI50—values correspond to the sample concentration achieving 50% of growth inhibition in human tumour cell lines or in liver primary culture PLP2. In each column different letters mean significant differences among total compounds ($p < 0.10$).

ultrasonic extractions and with 80% methanol and 70% acetone; and the analysis was performed using a LC–MS system. In this case, the results revealed a phenolic profile rich in gallic, *p*-coumaric, syringic, ferulic, vanillic, chlorogenic, rosmarinic and hydroxybenzoic acids; and in flavonoids such as quercetin, isoquercetin, kaempferol, neohesperidin and hesperidin. The total concentration in phenolic compounds ranged between 752.97 ± 5.04 and 875.84 ± 77.89 μg/g fresh weight.

Repo-Carrasco-Valencia, Hellström, Pihlava, & Mattila (2010) also studied the phenolic composition in six ecotypes of Andean indigenous quinoa, collected from the Agronomical Experimental Station-INIA Salcedo, Peru. The determination of flavonoids and phenolic acids was made using a HPLC system and the results revealed the presence of caffeic, ferulic, *p*-coumaric, benzoic, and vanillic acids, with a total of 37 ± 9 mg/100 g; and also the presence of several flavonoids, such as: myricetin, quercetin, kaempferol and isorhamnetin, with a concentration of 58 ± 13 mg/100 g. Another study performed on *Chenopodium quinoa* Willd was made by Tang, Zhang, Chen, Liu, & Tsao (2015), who identified the composition of different forms of extractable phenolics and betacyanins of quinoa cultivars of white, red and black seeds from Ontario, Canada. The extraction was made with acidified aqueous methanol and the compounds were identified using a HPLC system. The obtained results indicated the presence of 23 phenolic compounds, being the majority of them phenolic acids, mainly vanillic and ferulic acid and their derivatives. Among the main flavonoids, they identified quercetin, kaempferol and their glycosides. The total concentration of phenolic compounds was 466.99 ± 3.27, 634.66 ± 5.87 and 682.05 ± 4.73 mg/kg, for white, red and black varieties, respectively.

Finally, Multari, Marsol-Vall, Keskitalo, Yang, & Suomela (2018) studied the effects of different drying temperatures on the content of phenolic compounds in quinoa seeds from Jokioinen, Finland. The extraction procedure was made using an ultrasonic bath and with HCl and EtOHAc as solvents; and a UPLC-PDA-ESI-MS system for analysis of phenolic compounds. In general, the results evidenced the presence of vanillic, gallic, *p*-benzoic and ferulic acids, as well as syringaldehyde, quercetin and kaempferol. Ferulic acid and quercetin were the main phenolics identified in all of their samples. In all the aforementioned studies, several differences in the phenolic profile were observed. In some cases, other studies detected compounds other than those detected in the present study, and furthermore, the individual and total concentrations of polyphenols are very heterogeneous. This can arise from the different geographic locations, which lead to distinct environmental conditions, namely, climate, soil, among others, that consequently have a high impact on the chemical composition of the species. The postharvest treatments and different varieties of quinoa may also be causes that explain this variability of results.

3.2. Cytotoxic potential evaluated in hydroethanolic extract of quinoa grains

Regarding the cytotoxicity results obtained in this work (Table 2), the quinoa colour varieties studied did not show antiproliferative capacity in any tumour cell lines tested, producing GI_{50} values > 400 μg/mL. However, regarding the hepatotoxicity assay, none of the samples presented toxicity in the primary non-tumour cell culture tested (PLP2) (GI_{50} > 400 μg/mL). Statistically, there was no effect of quinoa colour variety on the cytotoxic incapacity of their extracts. In addition, it was evident that the consumption of black, red or white varieties do not represent any health hazard, being safe for incorporation in the daily diet.

So, in summary, this study of cytotoxic action showed that the absence of inhibition of the growth of tumour cell lines (GI_{50} > 400 μg/mL) means that extracts obtained from samples of different varieties of quinoa have no anti-tumour potential. In contrast, the fact that there is also an absence of inhibition in the culture of primary non-tumour cells, means that they do not present toxicity, as they allow the normal cell growth of a healthy culture. This shows that the consumption of these quinoa varieties does not represent a risk of toxicity.

Other authors, such as Nowak, Szewczyk, Gawlik-Dziki, Rzymowska, & Komsta (2016), studied the cytotoxic properties of lipophilic compounds extracted from different parts of other *Chenopodium* L. (*C. album*, *C. hybridum*, *C. rubrum* and *C. urbicum*) species and the extracts were assessed against human lung carcinoma A-549, ovarian carcinoma TOV-112D and normal human fibroblast cell lines. The results showed a significant antiproliferative effect on the TOV-112 cell line in the extracts from herb and seeds of *C. album* and *C. hybridum*. On the other hand, Gawlik-Dziki et al. (2013) explored the nutraceutical potential of quinoa leaves, namely, the

Table 3
Antibacterial activity (MIC and MBC, mg/mL) of *C. quinoa* Willd. grains hydroethanolic extract. The results are present as mean ± SE.

	B.c.		*S.a.*		*L.m.*	
	MIC	MBC	MIC	MBC	MIC	MBC
Black	0.107 ± 0.0161[a]	0.192 ± 0.0279[a]	0.572 ± 0.0854[a]	0.867 ± 0.1008[a]	0.472 ± 0.0805[a]	0.783 ± 0.1012[a]
Red	0.074 ± 0.0145[a]	0.153 ± 0.0253[a]	0.482 ± 0.0773[a]	0.739 ± 0.0912[a]	0.436 ± 0.0728[a]	0.682 ± 0.015[a]
White	0.111 ± 0.0111[a]	0.197 ± 0.0192[a]	0.592 ± 0.0588[a]	0.916 ± 0.0694[a]	0.547 ± 0.0554[a]	0.868 ± 0.0696[a]
Streptomycin	0.10 ± 0.01	0.20 ± 0.01	0.040 ± 0.001	0.10 ± 0.01	0.20 ± 0.01	0.30 ± 0.01
Ampicillin	**0.25 ± 0.02**	**0.40 ± 0.01**	**0.25 ± 0.01**	**0.45 ± 0.02**	**0.40 ± 0.01**	**0.50 ± 0.02**
Overall quinoa	0.099 ± 0.009	0.184 ± 0.015	0.556 ± 0.045	0.854 ± 0.054	0.499 ± 0.042	0.796 ± 0.054
	E.c.		*En.cl.*		*S.t.*	
	MIC	MBC	MIC	MBC	MIC	MBC
Black	0.300 ± 0.0616[a]	0.467 ± 0.0814[a]	0.439 ± 0.0797[a]	0.800 ± 0.1120[a]	0.533 ± 0.0810[a]	0.850 ± 0.0969[a]
Red	0.270 ± 0.0557[a]	0.409 ± 0.0737[a]	0.414 ± 0.0721[a]	0.709 ± 0.1013[a]	0.459 ± 0.0733[a]	0.682 ± 0.0876[a]
White	0.237 ± 0.0424[a]	0.355 ± 0.0560[a]	0.476 ± 0.0548[a]	0.726 ± 0.0771[a]	0,521 ± 0.0557[a]	0.845 ± 0.0667[a]
Streptomycin	0.20 ± 0.01	0.30 ± 0.01	0.20 ± 0.01	0.30 ± 0.02	0.20 ± 0.01	0.30 ± 0.01
Ampicillin	**0.40 ± 0.02**	**0.50 ± 0.02**	**0.25 ± 0.01**	**0.50 ± 0.01**	**0.75 ± 0.02**	1.20 ± 0.04
Overall quinoa	0.261 ± 0.032	0.396 ± 0.043	0.450 ± 0.041	0.738 ± 0.058	0.506 ± 0.042	0.800 ± 0.052

SE - standard error; MIC: Minimal Inhibitory Concentration; MBC: Minimal Bactericidal Concentration; S: Streptomycin; A: Ampicillin; B.c.: *Bacillus cereus*; S.a.: *Staphylococcus aureus*; L.m.: *Listeria monocytogenes*; E.c.: *Escherichia coli*; En. cl.: *Enterobacter cloacae*; S.t.: *Salmonella typhimirium*. In each column different letters mean significant differences among total compounds ($p < 0.10$).

phenolic content and its combined bioactivity. For the evaluation of the cytotoxic potential, an experimental model based on *in-vitro* cultivated prostate cancer (MAT-LyLu and AT-2 cell lines) was used, and the results pointed towards a high bioavailability of bioactive compounds, evidenced by their *in-vitro* effects on prostate cancer cells. Hu et al. (2017) studied the anticancer effects in polysaccharides obtained from the optimization of quinoa extraction, and the results revealed a significant proliferation inhibition against cancer cells in a dose- and time-dependent manner; yet, no remarkable influence in normal cells was observed. It is worth mentioning that, until now, no studies have been found to report the cytotoxic potential of quinoa grains in tumour and non-tumour cell lines, being all of the retrieved studies done only on the plant.

This cytotoxicity test comes with a scientific novelty, because it is the first to evaluate three different varieties of quinoa grains (black, red and white). Previous studies have only studied other parts of quinoa (for example leaves) or even other varieties of *Chenopodium*.

3.3. Antimicrobial activity evaluated in hydroethanolic extract of quinoa grains

The results of antibacterial and antifungal activities of quinoa extracts are presented in Tables 3 and 4, respectively. The samples were tested considering bacteria and fungi strains selected on the basis of their relevance to public health. For antibacterial analysis (Table 3), several Gram-positive and Gram-negative bacteria strains were used (*Bacillus cereus, Staphylococcus aureus, Listeria monocytogenes, Escherichia coli, Enterobacter cloacae* and *Salmonella* Typhimurium). The analysed extracts revealed antibacterial activity with inhibitory and bactericidal potential against all studied strains. The results of the samples showed MIC values between 0.107–0.867 mg/mL, 0.074–0.739 mg/mL and 0.111–0.592 mg/mL, for black, red and white varieties, respectively. All extracts showed better inhibitory efficacy against *B. cereus* with MIC values of 0.107 (black), 0.074 (red) and 0.111 mg/mL (white). On the other hand, the results revealed lower potential against *S. aureus,* with values of 0.867, 0.739 and 0.592 mg/mL for black, red and white varieties, respectively.

Regarding the bactericidal capacity, the values ranged between 0.192 and 0.867 mg/mL for the black variety, 0.153 and 0.739 mg/mL for the red variety, and 0.197 and 0.916 for the white variety. Once again, the *B. cereus* strain was more susceptible to all extracts, presenting lower MBC values (between 0.153 and 0.197 mg/mL). Contrarily, *S. aureus* presented more resistance to extracts, presenting hight MBC values (between 0.739 and 0.916 mg/mL).

To ensure the accuracy of the assay, standards controls (commercial antibiotics) were used - streptomycin and ampicillin. These antibiotics showed MIC values that ranged between 0.04–0.20 mg/mL and 0.25–0.75 mg/mL, respectively. Taking into account the MBC values, there is an oscillation between 0.10 and 0.30 mg/mL for streptomycin and between 0.40 and 1.20 mg/mL for ampicillin. In both cases, the bactericidal (MBC) and bacteriostatic (MIC) potential of the extracts closely approximates the values of the commercial antibiotics used. However, according to the statistical analysis, there are no significant differences between the varieties in their activity against the studied bacterial strains.

Considering the absence of statistically significant differences, a general average of the quinoa samples was performed for each bacterial activity (bactericidal - MBC and bacteriostatic - MIC), taking into account the different strains. This non-existence of statistical significance is due to the analysis of a large number of samples within each variety (black, red and white), which resulted in high standard errors. Thus, the MIC values varied between 0.099 ± 0.009 and 0.556 ± 0.045 mg/mL for the *Bacillus cereus* and *Staphylococcus aureus* strains, respectively; and the MBC values oscillated between 0.184 ± 0.015 and 0.854 ± 0.054 mg/mL for the same strains.

The antibacterial activity verified in this assay can be explained by the higher concentration of phenolic compounds found in the extracts;

Table 4
Antifungal activity (MIC and MFC, mg/mL) of *Chenopodium quinoa* Willd grains hydroethanolic extracts. The results are present as mean ± SE.

	A.fum		*A.o.*		*A.n.*	
	MIC	MFC	MIC	MFC	MIC	MFC
Black	0.417 ± 0.0714[a]	0.683 ± 0.0992[a]	0.137 ± 0.0344[a]	0.283 ± 0.0680[a]	0.567 ± 0.0685[a]	0.867 ± 0.0979[a]
Red	0.368 ± 0.0646[a]	0.668 ± 0.0897[a]	0.103 ± 0.0311[a]	0.211 ± 0.0615[a]	0.352 ± 0.06020[ab]	0.682 ± 0.0886[a]
White	0.464 ± 0.0491[a]	0.821 ± 0.0683[a]	0.176 ± 0.0237[a]	0.326 ± 0.0468[a]	0.500 ± 0.0471[a]	0.884 ± 0.0674[a]
Ketoconazole	0.25 ± 0.03	0.50 ± 0.02	0.20 ± 0.01	0.50 ± 0.03	0.20 ± 0.01	0.50 ± 0.01
Bifonazole	**0.15 ± 0.01**	**0.20 ± 0.01**	**0.10 ± 0.01**	**0.20 ± 0.03**	**0.15 ± 0.01**	**0.20 ± 0.02**
Overall quinoa	0.426 ± 0.038	0.746 ± 0.053	0.147 ± 0.019	0.284 ± 0.036	0.474 ± 0.038	0.823 ± 0.053
	P.o.		*P.fum.*		*P.v.c.*	
	MIC	MFC	MIC	MFC	MIC	MFC
Black	0.397 ± 0.0842[a]	0.683 ± 0.1359[a]	0.403 ± 0.0907[a]	0.717 ± 0.1420[a]	0.508 ± 0.0866[a]	0.850 ± 0.1311[a]
Red	0.309 ± 0.0761[a]	0.641 ± 0.1229[a]	0.300 ± 0.820[a]	0.505 ± 0.1284[a]	0.361 ± 0.0783[a]	0.723 ± 0.1185[a]
White	0.447 ± 0.0579[a]	0.778 ± 0.0935[a]	0.439 ± 0.0624[a]	0.725 ± 0.0977[a]	0.471 ± 0.0596[a]	0.861 ± 0.0902[a]
Ketoconazole	2.50 ± 0.04	3.50 ± 0.04	0.20 ± 0.02	0.50 ± 0.01	0.20 ± 0.01	0.30 ± 0.03
Bifonazole	**0.20 ± 0.01**	**0.25 ± 0.01**	**0.20 ± 0.01**	**0.25 ± 0.02**	**0.10 ± 0.01**	0.20 ± 0.01
Overall quinoa	0.397 ± 0.045	0.717 ± 0.071	0.392 ± 0.048	0.661 ± 0.075	0.449 ± 0.046	0.819 ± 0.069

SE - standard error; MIC: Minimal Inhibitory Concentration; MFC: Minimal Fungicide Concentration; K: Ketoconazole; B: Bifonazole; A.fum.: *Aspergillus fumigatus*; A.o.: *Aspergillus ochraceus*; A.n.: *Aspergillus niger*; P.fum.: *Penicillium funiculosum*; P.o.: *Penicillium ochrochloron*; P.v.c.: *Penicillium verrucosum* var. *cyclopium*. In each column different letters mean significant differences among total compounds ($p < 0.10$).

nevertheless, other non-identified compounds in the extracts may also contribute to this bioactivity.

With regards to the antifungal activity (Table 4), several strains were used, such as: *Aspergillus fumigatus*, *Aspergillus ochraceus*, *Aspergillus niger*, *Penicillium funiculosum*, *Penicillium ochrochloron* and *Penicillium verrucosum* var. *cyclopium*. This assay evidenced the antifungal potential in extracts of the three quinoa varieties, with MIC values oscillating between 0.103 and 0.567 mg/mL; and MIC values between 0.211 and 0.884 mg/mL. The extract of the quinoa black variety revealed a promising inhibitory capacity, with MIC values of 0.137 and 0.567 mg/mL against *Aspergillus ochraceus* and *Aspergillus niger,* respectively. Also, the extract of the quinoa red variety exhibited the same potential, presenting MIC values of 0.103 and 0.368 mg/mL against *Aspergillus ochraceus* and *Aspergillus fumigatus*, respectively. Finally, for the white variety, the MIC values ranged between 0.176 and 0.500 mg/mL against *Aspergillus ochraceus* and *Aspergillus niger*, respectively. Thus, it was evident that *Aspergillus ochraceus* was the most sensitive strain to all studied extracts. By contrast, the strains that showed the greatest resistance were *Aspergillus niger* and *Aspergillus fumigatus.*

Regarding the fungicidal capacity of the extracts, MFC values ranged between 0.283 and 0.867 mg/mL, 0.211 and 0.723 mg/mL, and 0.326 and 0.884 mg/mL for black, red and white varieties, respectively. As observed with the MIC values, *Aspergillus ochraceus* stood out as the strain with the most promising results, with MFC values of 0.283, 0.211 and 0.326 mg/mL for black, red and white varieties, respectively. *Aspergillus niger* and *Penicillium verrucosum* var. *cyclopium* were found to have the greatest fungicidal resistance, showing higher MFC values in comparison to the other strains.

Commercial antifungal drugs ketoconazole and bifonazole were employed as positive controls. Taking into account the inhibitory potential, these compounds presented MIC values that ranged between 0.20 and 2.50 mg/mL, and between 0.10 and 0.20 mg/mL for ketoconazole and bifonazole, respectively. Regarding the fungicidal capacity, these compounds showed MFC values of 0.30–3.50 mg/mL (ketoconazole) and 0.20–0.25 mg/mL (bifonazole). In all cases, the values obtained for the quinoa extracts approximated the lowest values obtained with the control antifungal compounds, revealing favourable results for all varieties of the quinoa grains. Regarding the statistical analysis, in this assay, there are no significant differences between the colour varieties in each of the studied microorganisms.

Also, in the antifungal activity, the results obtained can be justified by the presence of phenolic compounds found in the different extracts, or other non-identified bioactive molecules. To date, no studies have been carried out on the antimicrobial potential of extracts of quinoa grains of these varieties. This study has hence revealed another benefit of the use of this matrix, which enhances its value as a functional food.

The study of antimicrobial activity (antibacterial and antifungal) also emerges as a novelty in this work, in the detection of bioactive properties of different varieties of quinoa grains. A bibliographic review was made by the authors, and no data were found on this biological potential of quinoa grains.

As in the study of antibacterial activity, also in the study of antifungal activity there no statistical differences between colour varieties could be found. Instead, a high variability (as inferred from the high standard errors) in antibacterial activity within each colour variety was found. For that reason, general means of the fungicide - MFC and fungistatic – MIC properties for each fungal strain were computed. MIC values varied between 0.147 ± 0.019 (*Aspergillus ochraceus*) and 0.474 ± 0.038 mg/mL (*Aspergillus niger*), and the MFC values varied between 0.284 ± 0.036 (*Aspergillus ochraceus*) and 0.823 ± 0.053 mg/mL (*Aspergillus niger*).

In order to relate the antimicrobial activity obtained in this study with the different compounds detected in the quinoa samples, a correlation analysis was carried out. The results are presented in Table 5. The highest coefficient of correlations (R) presented values of approximately 0.40. These moderate correlations occurred for the

Table 5
Correlation study between the different phenolic compounds identified in *C. quinoa* Willd. varieties (peaks 1–8) and the bactericidal (MBC), bacteriostatic (MIC), fungicidal (MFC) and fungistatic (MIC) activities for each strain. Numbers closer to 1 indicate higher positive correlation while those closer to −1 higher negative correlation.

	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8
MIC B.*c.*	−0.08	0.13	0.36	−0.40	−0.21	0.40	−0.26	0.31
MIC *S.a.*	−0.15	−0.05	0.08	−0.15	0.03	0.17	0.10	0.13
MIC *L.m.*	−0.19	−0.10	0.13	−0.23	−0.10	0.21	0.03	0.20
MIC *E.c.*	0.20	0.04	−0.10	0.02	0.03	0.03	0.17	−0.04
MIC *Em.cl.*	−0.12	−0.02	0.06	−0.21	−0.10	0.10	−0.09	0.07
MIC *S.t.*	−0.01	0.06	0.04	−0.19	−0.10	0.07	−0.07	0.02
MBC B.*c*	−0.11	0.08	0.31	−0.26	−0.06	0.39	−0.10	0.31
MBC *S.a*	−0.17	−0.06	0.09	−0.20	−0.03	0.14	0.03	0.12
MBC *L.m.*	−0.14	−0.15	0.11	−0.19	−0.11	0.18	0.10	0.21
MBC *E.c.*	0.23	0.09	−0.12	0.02	0.02	−0.01	0.15	−0.09
MBC *Em.cl.*	0.02	0.10	−0.08	−0.09	−0.01	−0.01	−0.01	−0.05
MBC *S.t.*	0.08	0.12	0.12	−0.29	−0.18	0.09	−0.18	0.06
MIC *A.fum*	−0.10	0.12	0.12	−0.12	−0.04	0.07	−0.22	0.01
MIC *A.o.*	−0.25	0.03	0.40	−0.24	−0.15	0.39	−0.13	0.33
MIC *A.n.*	−0.09	0.34	0.16	−0.34	−0.27	0.09	−0.32	−0.03
MIC *P.o.*	−0.23	0.01	0.27	−0.27	−0.18	0.30	−0.06	0.25
MIC *P.fum.*	−0.20	0.09	0.26	−0.31	−0.18	0.24	−0.18	0.22
MIC *P.v.c.*	−0.06	0.19	0.26	−0.32	−0.19	0.25	−0.17	0.17
MFC *A.fum*	−0.13	0.05	0.09	−0.05	0.02	0.05	−0.17	0.00
MFC *A.o.*	−0.18	0.11	0.29	−0.16	−0.06	0.28	−0.11	0.23
MFC *A.n.*	−0.24	0.15	0.21	−0.29	−0.20	0.20	−0.18	0.12
MFC *P.o.*	−0.26	0.00	0.15	−0.16	−0.07	0.18	0.01	0.18
MFC *P.fum.*	−0.28	0.10	0.20	−0.25	−0.16	0.16	−0.13	0.13
MFC *P.v.c.*	−0.10	0.09	0.20	−0.22	−0.12	0.22	−0.06	0.18

MIC: Minimal Inhibitory Concentration; MBC: Minimal Bactericidal Concentration; MFC: Minimal Fungicide Concentration; B.c.: *Bacillus cereus*; S.a.: *Staphylococcus aureus*; L.m.: *Listeria monocytogenes*; E.c.: *Escherichia coli*; En. cl.: *Enterobacter cloacae*; S.t.: *Salmonella typhimirium*; A.fum.: *Aspergillus fumigatus*; A.o.: *Aspergillus ochraceus*; A.n.: *Aspergillus niger*; P.fum.: *Penicillium funiculosum*; P.o.: *Penicillium ochrochloron*; P.v.c.: *Penicillium verrucosum* var. *cyclopium*.

following variables: peak 3 with MIC *A.o.* and peak 6 with MIC *B.c.* Furthermore, the R coefficients between peak 6 and MBC *B.c.*, peak 6 and MIC *A.o.*, and peak 3 with MIC *B.c.* also showed low to moderate correlation with values of 0.39, 0.38 and 0.36, respectively. This study did not evidence strong correlations between phenolic compounds and antimicrobial activity. It is likely that a greater sample size for each quinoa variety would be needed in order to elucidate stronger associations.

4. Conclusion

Chenopodium quinoa Willd. grains constitute a food matrix increasingly cultivated, appreciated and consumed in different parts of the world. In this sense, it is essential to carry out studies that seek to enlarge the knowledge on its composition and benefits of its consumption. Analyses of samples of white, red and black quinoa seeds revealed a rich phenolic profile, especially in flavonoids, which are able to act beneficially on consumer's health. Furthermore, the absence of toxicity of the seeds, proven in this study, allows endorsing quinoa as a safe food for daily consumption. The antimicrobial activity assays also produced very promising values, considering the antibacterial and antifungal capacity of the quinoa extracts. However, in general, no quinoa variety (black, red and white) can be highlighted in relation to this bioactivity study, since there were no statistically significant differences between the quinoa varieties studied in terms of bactericidal, bacteriostatic, fungicidal and fungistatic activities. Thus, the pioneer studies carried out in this work, namely the bioactive potential of different varieties of quinoa grains, allowed to make known the promising antimicrobial capacity of this pseudocereal, as well as the absence of its toxicity. In conclusion, quinoa is valuable not only for its high nutritional potential, but also for its good composition in bioactive compounds.

CRediT authorship contribution statement

Eliana Pereira: Methodology, Investigation, Writing - original draft. **Vasco Cadavez:** Methodology, Writing - original draft. **Lillian Barros:** Conceptualization, Methodology, Writing - review & editing. **Christian Encina-Zelada:** Methodology. **Dejan Stojković:** Methodology, Writing - original draft. **Marina Sokovic:** Methodology, Writing - review & editing. **Ricardo C. Calhelha:** Methodology. **Ursula Gonzales-Barron:** Conceptualization, Methodology, Writing - review & editing. **Isabel C.F.R. Ferreira:** Conceptualization, Methodology, Writing - review & editing.

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.

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