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Original Article

The Phytochemical Content and Cytotoxic Effects of Quinoa Commercial Cultivars (*Chenopodium quinoa* Willd.) on MCF7 Cell Line of Breast Cancer

Running title: **Total Phenolic, Flavonoid Content, Antioxidant and Cytotoxic Effects of Seed and Leaf Extracts of Some Quinoa Commercial Cultivars (*Chenopodium quinoa* Willd.) on MCF7 Cell Line of Breast Cancer**

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ABSTRACT

The Black, Titicaca, and Multi-hued Bulk cultivars were cultivated in the arid and semi-arid regions of Iran to compare the phenolic compounds, antioxidant, and cytotoxic effects of seed and leave extracts of Quinoa on the MCF7 cell line of breast cancer (at 100, 250, 500, and 1000 mg/mL concentrations). The bioactive compounds of samples were extracted using the hydroethanolic solvent using the Soxhlet method. According to this research, although the Titicaca seed extract had the highest total phenolic content at 1000 mg/mL (152 mgGAE/g), the highest average phenolic content was observed in the leaf extracts of all cultivars. So, the seed extract of the Black cultivar showed the highest flavonoid content at 1000 mg/mL (268 mg QE/gDW). The most increased DPPH radical scavenging activity was observed in the leaf extract of the Black cultivar (54.23%), and the highest ferric-reducing power was exhibited by Titicaca leaf extract at 1000 mg/mL (0.37 mmol Fe^{2+}). The results indicated that the leaf extract of the Black cultivar had the lowest IC_{50} value (786.95 mg/mL). Based on the results, the leaf and seed extracts of Black and Titicaca cultivars were selected to examine the cytotoxic effects of Quinoa extracts on the MCF7 cell line due to their higher phenolic and antioxidant contents. The lowest MCF7 cell line viability percentage (13.92%) was observed in the leaf extract of the Black cultivar at 1000 mg/mL concentration after 72 hours. Regardless of the extract concentration, the leaf and Black cultivar were the superior organ and cultivar compared to other studied characteristics, respectively. In addition, 72 hours significantly yielded better results than other periods.

Keywords: Black Quinoa, DPPH, MCF7 Cell Line, MTT assay

INTRODUCTION

Cancer is a leading cause of death in developing countries, exponentially developing due to environmental and geographical factors, population aging, and rising risk factors such as physical inactivity, tobacco use, urbanization, and economic development [1, 2]. Iran is experiencing a high rate of cancer growth so that cancer is projected to become the second leading cause of death in the country by 2025. Studies in geographical regions of Iran have indicated that the longitudinal extension increased cancer rates significantly. In the vast deserts of Iran, like the Lūt Desert in the eastern regions and the low-lying areas of the Iranian plateau in the east and southeast, the amount of wave received by the sun in the electromagnetic spectrum remains more on the ground due to the high thickness of the atmosphere in these regions. Moreover, the water vapor scarcity allows shorter waves to reach the ground, possibly contributing to the prevalence of breast cancer in the central lowlands and southeastern low-altitude areas [3].

Despite extensive medical advancements [4], the incidence rate of breast cancer has alarmingly risen over the past four decades as the second most common and deadly malignancy in women [5]. Potentially, one in every 10 to 15 women may develop this cancer in Iran [6]. A various array of approaches exists for breast cancer treatment,

including surgical interventions to remove tumors, radiation to destroy tumor tissues, and chemotherapy, whereby chemically formulated compounds are used to eliminate cancer cells. Given the concerns regarding drug resistance, cancer recurrence, and adverse side effects of conventional chemotherapy medications [7], researchers are investigating more efficacious treatments focusing on the potential of natural chemical compounds to combat breast cancer. The proponents of natural compounds contend that they offer a safer therapeutic approach, characterized by diminished toxicity, in treating breast cancer [7, 8].

Quinoa, initially domesticated in South America around 7,000 years ago, belongs to the Amaranthaceae family and Chenopodiaceae subfamily. This plant has gained extensive attention in the past decade for its agronomic, phytochemical, and overall nutritional properties (Chenopodium quinoa Willd.). Quinoa is known as the "Mother grain of the Incas" due to the exceptional properties of its seeds [9, 10]. The seed, the central edible part of Quinoa, is gluten-free and rich in proteins, essential amino acids, minerals, and vital vitamins, making it a "Super food" [10].

Quinoa seeds exhibit low saponin concentration and notable antibacterial and antioxidant effects for their high protein levels, carbohydrates, fiber, iron, total phenol, and flavonoid content [11, 12]. Furthermore, the antimicrobial, anticancer, antidiabetic, anti-obesity, and antioxidant effects of green parts of Quinoa, such as leaves, sprouts, and microgreens, have made it a valuable source of nutrients with significant biological effects for humans. The Incas and South American Indians extensively used Quinoa seeds and leaves in their diet to compensate for the lack of animal proteins [13, 14].

The bioactive components in Quinoa are widely regarded as exceptional natural antioxidants, attracting the attention of considerable scientific interest due to their notable benefits, such as safety, high efficiency, and absence of adverse effects, leading to comparisons with synthetic antioxidants, indicating the advantageous nature of quinoa-derived antioxidants. The ethyl acetate content of Quinoa seeds has the potential to generate natural antioxidants [15], polysaccharides [16], and saponins [17], highlighting positive effects on MCF7 breast cancer cell lines. The secondary metabolites in the plant’s seeds and shoots can protect cells against the negative effects of free radicals by slowing down or preventing the oxidation of molecules [18].

This study aimed to compare the phenolic, flavonoid, and antioxidant compositions of extracts from seeds and leaves of three commercially available and accessible Quinoa cultivars in Iran, followed by assessing the cytotoxic effects of two selected cultivars on the MCF7 breast cancer line. The cultivars under study were initially cultivated in the field conditions of the arid and semi-arid climate of Yazd to provide researchers with the opportunity to associate specific anticancer results with the randomly cultivated samples from a particular cultivation area.

MATERIALS AND METHODS

Plant Materials

In this study, the Black (with black color) [19], Titicaca (with yellow color) [20], and Multi-hued Bulk (with yellow color) [21] cultivars were (prepared from the Seed and Plant Improvement Institute of Karaj, Iran) cultivated in the arid and semi-arid climate of Yazd (Pilot Research Filed of Yazd University, located at 54°21'35" longitude and 31°49'40" latitude) in an experiment structured as a randomized complete block design with four replications (Figure 1 A). Some phytochemical traits were measured using random sampling of seeds and leaves of the studied cultivars (Figure 1B), considering the repetitions in the field. The samples were washed twice with distilled water, air-dried, and ground to a particle size of approximately 500 µm. About 30 g of the powdered sample was extracted with 300 mm of 80:20 hydro-ethanolic solvent to extract bioactive compounds [22] using the Soxhlet method (Figure 1C) [23]. Sterile and autoclaved vessels were employed in the extraction process to prevent microbial contamination. Later, the extracts were dried under completely sterile conditions, avoiding environmental contamination. Finally, the extract concentrations of 100, 250, 500, and 1000 mg/mL were prepared in three replications for further investigations.

Total Phenol Content

The phenolic compound content was tested using the Sonald and Laima 1999 method [24]. About 1mm of various extract concentrations was combined with 1 mm of 95% ethanol, and the solution volume was brought to 5 mL via double-distilled water. Subsequently, 0.5 mL of 50% Folin's reagent and 1 mL of 5% sodium carbonate were

added. The resulting mixture was stored in darkness for one hour. In the following, the absorbance of the sample at 720 nm was measured using a spectrophotometer (AnalytikJena specord 210, Analytik Jena GmbH+Co. KG, Germany). A standard curve was plotted employing different concentrations of gallic acid, and the total phenol content of the extracts was expressed in milligrams of gallic acid equivalent per gram of dry weight of the plant (mgGAE/gDW).

Fig. 1 A: Cultivation of studied cultivars in Yazd University research field, **B**: Labeling of randomly selected plants to measure the studied characteristics, **C**: Extraction of plant samples by Soxhlet method, and **D**: Preparation of samples to measure DPPH free radical inhibitory activity

Total Flavonoid Content

The total flavonoid content in various extract concentrations of the studied treatments was measured using the aluminum chloride colorimetric method [25]. As a result, 1 mm of the prepared extract concentrations, 75 µL of sodium nitrate (50% w/v), 0.15 mL of 10% aluminum chloride, and 0.5 mL of 1 Molar sodium hydroxide were added. Finally, the volume was increased to 2.5 mL using distilled water. The absorbance of the samples at 507 nm was determined using a spectrophotometer after 5 minutes. A standard curve was plotted using different concentrations of quercetin, and the total flavonoid content was expressed in milligrams of quercetin per gram of dry weight of the plant (mgQ/g DW).

DPPH Free Radical Scavenging

About 1 mL of various extract concentrations was mixed with 2 mL of 0.1 mm The 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution and kept for 30 minutes at room temperature in the dark to measure the antioxidant activity of the studied extracts using the DPPH free radical scavenging method (Figure 1D). Subsequently, the absorbance of the samples was spectrophotometrically read at a wavelength of 517 nm [27]. Butylated hydroxytoluene (BHT) was used as a standard antioxidant at concentrations similar to the extracts to compare the antioxidant activity of the extracts. Finally, the percentage of DPPH free radical scavenging by the extracts was calculated using the following formula.

$$\text{DPPH free radical scavenging\%} = \frac{A_B - A_S}{A_B} \times 100$$

In which, A_B shows control absorption and A_S presents sample absorption.

Ferric Reducing Antioxidant Power

The extract treatments' ferric-reducing antioxidant power (FRAP) was measured using the Oyaizu method [26]. About 1 mL of the extract, 1 mL of 0.5 Molar phosphate buffer (pH=6.6), and 1 mL of 10 mg/mL potassium ferrocyanide were mixed and incubated at 50℃ for 20 minutes. Next, 1 mL of trichloroacetic acid (TCA) was added to the mixture and centrifuged at 3000 revolutions per minute for 5 minutes. Finally, 1 mL of the supernatant was mixed with 1 mL of distilled water and 100 µL of 1% ferric chloride, and the absorbance at 700 nm (in terms of mmol Fe^{2+}) was read using a spectrophotometer. An increased absorption in the reaction mixture indicates an increase in the reducing power.

Preparation of Cell Culture from MCF7 Cell Line

MCF7 breast cancer cell line was obtained from Yazd Research Institute of Reproductive Sciences. To cultivate this cell line, pen-Strep, and Amphotericin-B were cultured at 37°C with 5% carbon dioxide and 96% humidity from the complete culture medium, including RPMI 1640 and 10% fetal bovine serum [28].

Cytotoxic Effect of Quinoa Extract on MCF7 Cell Line

The measurement was performed followed by placing the extracts with different concentrations near the cell line and evaluating the number of dead cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method. First, the percentage of live cells was checked by the Trypan Blue method. About 1×104 cells and 200 µL of culture medium were transferred to a 96-well plate and kept in an incubator for 24 hours. Cancer cells were treated with 180 µL of the culture medium containing 10% fetal bovine serum and 20 µL of extract at 100, 250, 500, and 1000 mg/mL after emptying the wells.

Later, the plates were transferred to the incubator and treated with MTT compound after 24, 48, and 72 hours of incubation. The culture medium of each well was entirely and cautiously drained in the first plate after 24 hours of incubation, and 100 µL of culture medium and 10 µL of MTT were added to each well. The plate containing the cells was kept in the incubator for 3 hours after adding MTT. In the following, the MTT content of all wells was drained, and 50 µL of Dimethylsulfoxide (DMSO) was pipetted into each well and placed in the incubator for 10 minutes. The plate was placed in the incubator shaker for 30 seconds to spread the purple color in the wells evenly. The optical absorbance of the wells was read by a BioTecELx800 model ELISA reader at a wavelength of 570 nm. According to Figure 2, these steps were performed for other plates after 48 and 72 hours of incubation, and the MTT solution was prepared using the BIO-IDEA kit (Idezist Notarkib Company, Iran).

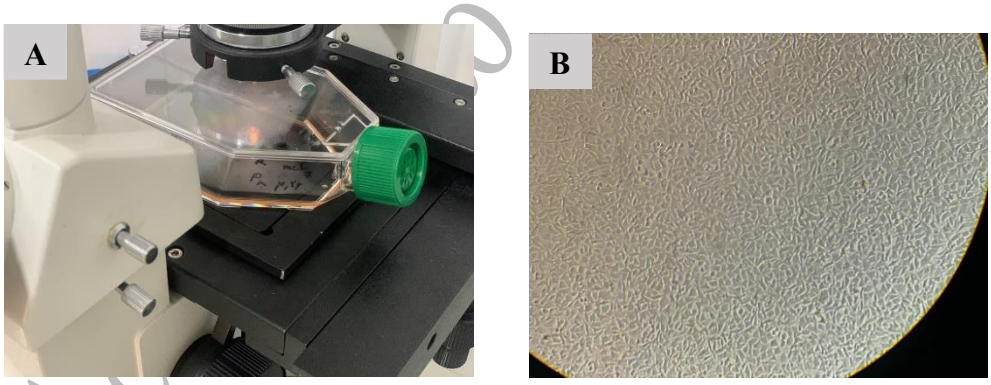

Fig. 2 A: Flask containing MCF7 cell line studied using an Elizarider device and **B**: MCF7 cell line under the microscope, scale bars=200 µm.

Statistical Analysis

Initially, the normality of the data was checked using the Kolmogorov-Smirnov test, and the homogeneity of variances was assessed by Bartlett-Levene's test. The measurement of phytochemical properties was analyzed using SPSS software version 26 by the factorial test method based on the completely randomized design in three replications after confirming the data normality and homogeneity. The investigated factors included plant sample (seed or leaf) and extract concentration (100, 250, 500, and 1000 mg/mL) for Black, Titicaca, and Multi-hued Bulk cultivars. Black and Titicaca cultivars were chosen to study the effect of Quinoa extract toxicity on the MCF7 breast cancer cell line because of their higher phenolic and antioxidant content.

Thus, the cytotoxic effect of seed and/or leaf extract (plant sample) of Black and Titicaca cultivars was analyzed in three replications in three time periods of 24, 48, and 72 hours in different concentrations of 100, 250, 500, and 1000 mg/mL based on factorial experiment and completely randomized design using $SPSS_{26}$.
Mean scores were compared using Duncan's multiple range test at a 5% probability level for each of the above studies, and graphs were plotted in Excel 2013 software.

RESULTS AND DISCUSSION

Phytochemical Analyses

The analysis of variance (ANOVA) of phytochemical traits indicated significant differences at 1% and 5% levels regarding cultivars, plant sample types, extract concentrations, and interaction effects (Table 1).

Table 1 ANOVA results of phytochemical traits of leaf and seed extracts of the studied Quinoa cultivar under different concentrations

Sources of variation	df	Mean of square			
		Total phenol (mgGAE/gDW)	Total flavonoid (mgQ/gDW)	DPPH scavenging (%)	FRAP (mmol Fe^{2+})
Cultivar (cv)	2	15208.53 **	17321.80 **	1373.58 **	0.17 **
Sample type (St)	1	4857.09 **	27635.39 **	3290.48 **	0.18 **
Concentration of extract (Ce)	3	24624.02 **	145035.37 **	4863.04 **	0.11 **
cv*St	2	900.84 **	1406.21 **	713.22 **	0.15 **
cv*Ce	6	2229.91 **	3142.87 **	122.39 **	0.053 **
St*Ce	3	485.70 **	10934.43 **	685.09 **	0.04 *
cv*St*Ce	6	481.94 **	656.94 **	93.20 **	0.05 **
Error	32	51.34	133.08	6.37	0.01
R^2		0.97	0.97	0.98	0.98

* and ** indicate significant difference at the 5% and 1% probability levels, respectively.

Total Phenol Content

Based on the mean data comparison, the highest phenolic content was observed in the leaf extract of three cultivars. The concentration of 1000 mg/mL seed extract (152.50 mgGAE/gDW) of the Titicaca cultivar showed the highest total phenolic content, which was not significantly different (*P-value*>0.05) from the phenolic content of the leaf extract of the same cultivar (145.61 mgGAE/gDW). Hence, the corresponding amount of total phenol in Black and Multi-hued Bulk cultivars was significantly higher in leaf samples than in seeds. The highest phenolic content in these two cultivars was observed at a concentration of 1000 mg/mL of leaf extract with 103.39 and 78.61 mgGAE/gDW of dry matter, respectively (Figure 3).

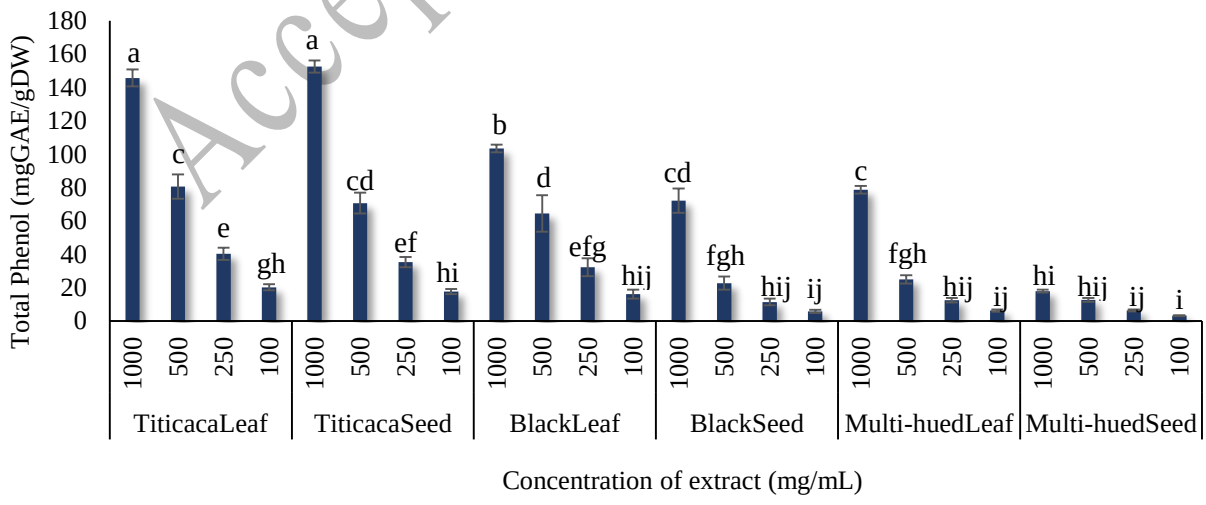

Fig. 3 Total phenolic content of different concentrations of leaf and seed extractions regarding the studied Quinoa cultivars. The columns with a letter in common are not significantly different at P-value≤ 0.05

Researchers observed higher polyphenolic content in bran, leaf, and root extract compared to Quinoa seed extract [29]. Regarding the phenolic, antioxidant, and anticancer properties of Puno and Titicaca Quinoa cultivars, the Titicaca cultivar showed a higher phenolic content than the other cultivars and 13 phenolic compounds, including five phenolic acid compounds, seven flavonoid compounds, and one pterostilbene compound were identified [30]. Accordingly, Pellegrini *et al*. (2018) found that white Quinoa had a higher total phenolic content than black Quinoa [31].

Another research on the seeds of 30 colored Quinoa cultivars studied the betalain content in edible Quinoa seeds and the possible relationship between the presence of pigments and antioxidants and the free radical scavenging capacity of the seeds. The highest total phenol content was reported in black Quinoa JQ-00145 (569.25 mgGAE/100g) and red Quinoa JQ-00125 (348.08 mgGAE/100g), respectively [32]. However, Liu found that the phenolics in Quinoa were present in free and bound forms, and the phenolic characteristics of Quinoa seeds were different depending on their color. According to this study, the total phenol content in white Quinoa was mainly free and bound in red and black Quinoa [33]. The difference in free phenolic acids among Quinoa cultivars in different studies may be due to the cultivar's diversity and the crushing level of plant samples before extraction [34].

Total Flavonoid Content

Contrary to the total phenolic content results, the highest flavonoid content was related to the seed extracts compared to the leaf extracts of different studied Quinoa cultivars. Black cultivar had the highest flavonoid content in extracting 1000 mg/mL seeds (268.83 mgQ/gDW), which was not significantly different from the flavonoid content in the extraction of 1000 mg/mL seeds of Titicaca cultivar (255.17 mgQ/gDW). The lowest amount of total flavonoid was obtained from extracting the Black cultivar seed at a concentration of 100 mg/mL (3.04 mgQ/gDW), showing no significant difference with the leaf extract of the same cultivar as well as with the seed and leaf extract of Titicaca and Multi-hued Bulk cultivars at a concentration of 100 mg/mL of the extract (Figure 4).

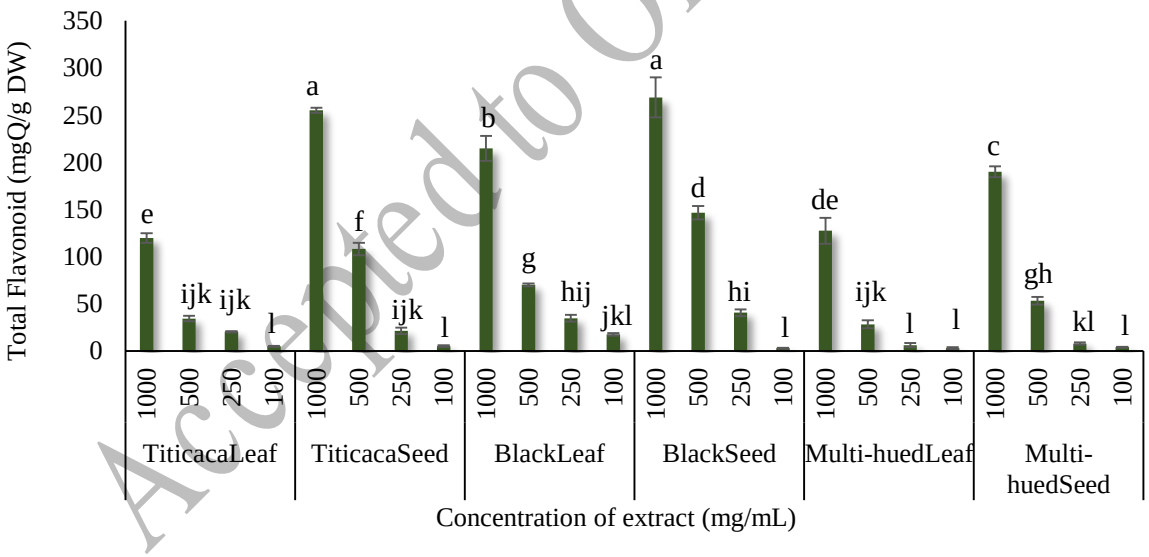

Fig. 4 Total flavonoid content of different concentrations of leaf and seed extractions of the studied Quinoa cultivars. The columns with a letter in common are not significantly different at P-value≤ 0.05

A study was conducted to evaluate the phenolic, antioxidant, anti-inflammatory, and antitumor compounds of white (BLWY), red (BLWY), and black (BL) Quinoa lines originating from Bolivia and Shanxi Province, China. The flavonoid content was higher in red and black samples than in white, indicating that the amount of flavonoid was directly related to the grain color. The number of flavonoids increased as the color turned into darker shades. The amount of flavonoid and color of Quinoa seed was related to the expression level of key genes in flavonoid biosynthesis [35]. These results were consistent with the literature, indicating the high amount of flavonoid in colored Quinoa cultivars compared to white Quinoa [36-38]. In investigating the phenolic and antioxidant

properties of Quinoa, the highest total flavonoid content was observed in root extract (54.14 mgQ/gDW), followed by leaf extract (51.29 mgQ/gDW), seed extract (45.88 mgQ/gDW), and Quinoa stem extract (42.07 mgQ/gDW) [29]. In addition to the cultivar and plant sample, the number of polyphenol compounds (i.e., phenolic acids and flavonoids) is greatly influenced by environmental conditions, soil, plant age, harvest, and post-harvest conditions [31].

DPPH Free Radical Scavenging

Phenolic antioxidants in Quinoa seeds exist either freely or bound to cell wall structures [39]. The mean comparison of the data showed that the highest ability to inhibit DPPH free radicals was related to the Black cultivar leaf extract at 1000 mg/mL (54.23), which did not show a significant difference with 500 mg/mL of this extract. The inhibitory level of DPPH free radicals in the leaf and seed extracts of Titicaca and Multi-hued Bulk cultivars was significantly (*P-value*<0.01) lower than that of the Black cultivar in the corresponding samples and concentrations. However, the lowest free radical inhibitory value was observed in the concentration of 1000 mg/mL of Multi-hued Bulk leaf extract (3.70). Similar to the Black cultivar, the highest free radical inhibitory value was obtained in the leaf sample of two other studied cultivars at 1000 mg/mL for Titicaca and Multi-hued Bulk cultivars (49.85 and 42.70), respectively (Figure 5). These results were consistent with the three cultivars' total phenolic and flavonoid content investigated in the present study (Figures 3 and 4).

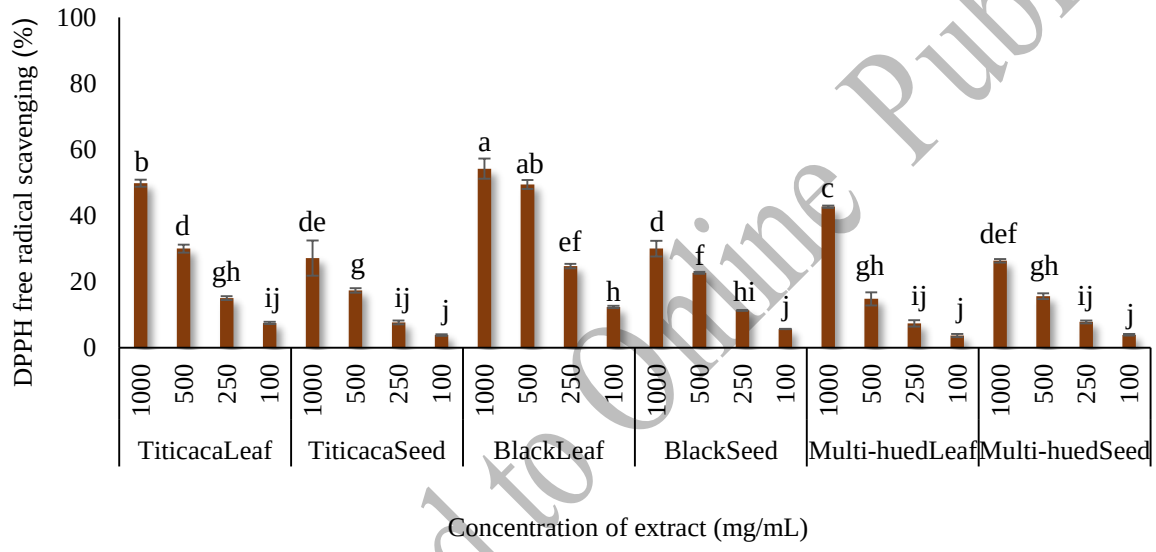

Fig. 5 DPPH free radical scavenging of different concentrations of leaf and seed extractions of the studied Quinoa cultivars. The columns with a letter in common are not significantly different at P-value≤ 0.05

In a study on the antioxidant content of 30 colored Quinoa cultivars, researchers stated that black Quinoa JQ-00145 had the highest antioxidant activity [32]. Accordingly, Pellegrini *et al*. reported more antioxidants in black and red Quinoa than white Quinoa [31]. According to the related literature, differences exist among different Quinoa cultivars regarding phytochemical properties, such that cultivars with dark Quinoa seeds showed higher antioxidant effects than cultivars with light seeds [33, 37]. The increase in total phenolic content may be metabolically related to betalain content in purple, red, and yellow Quinoa seeds [40]. However, the bound phenols in white Quinoa can show significantly higher DPPH radical scavenging ability than in black or red Quinoa [41].

On the other hand, antioxidant capacity can be measured using the inhibitory concentration (IC_{50}). The IC_{50} required to achieve 50% antioxidant capacity is calculated as DPPH. Accordingly, a lower IC_{50} increases the obtained free radical scavenging activity. Figure 6 illustrates the IC_{50} of leaf and seed extracts of the studied Quinoa cultivars at different concentrations. Black cultivar leaf extract showed the lowest IC_{50} value (786.95

mg/mL). In addition, the highest value of IC_{50} was related to the seed extract of the Multi-hued Bulk cultivar (4929.00 mg/mL).

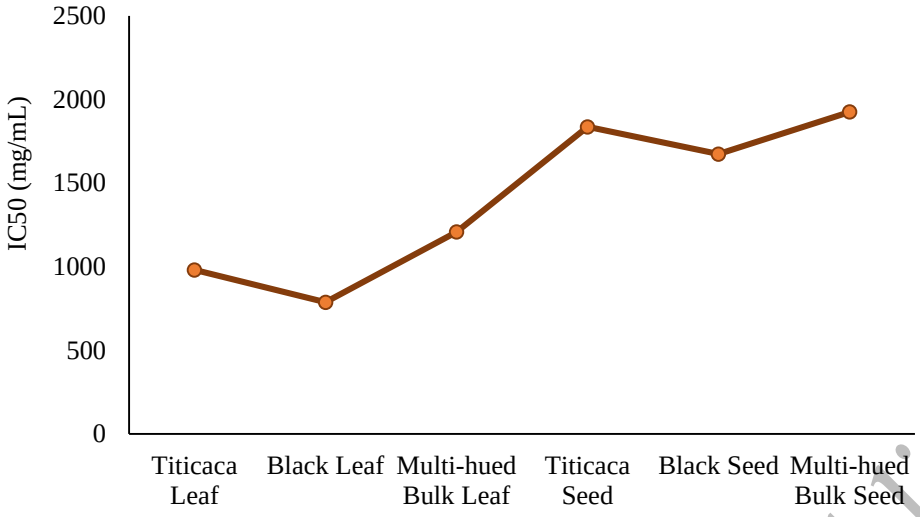

Fig. 6 DPPH IC_{50} (mg/mL) of leaf and seed extracts of studied Quinoa cultivars

Ferric Reducing Antioxidant Power

The highest FRAP was related to Titicaca leaf extract at 1000 mg/mL (0.37 mmol Fe^{2+}), which was not significantly different from the seed extract of the same cultivar at the same concentration (0.32 mmol Fe^{2+}) (*P-value>0.05*). The FRAP can vary depending on the type of plant organ because the seed extract of the Black cultivar (0.28 mmol Fe^{2+}) and the leaves of the Multi-hued Bulk cultivar (0.27 mmol Fe^{2+}) at 1000 mg/mL showed more FRAP following the Titicaca cultivar. This result may be due to the difference in the content of their phenolic compounds. The results indicated a significant decrease (*P-value<0.05*) in FRAP with decreasing extract concentration in the studied plant samples and cultivars. The lowest value of this characteristic was observed at 100 mg/mL of the Black leaf extract (0.02 mmol Fe^{2+}) (Figure 7).

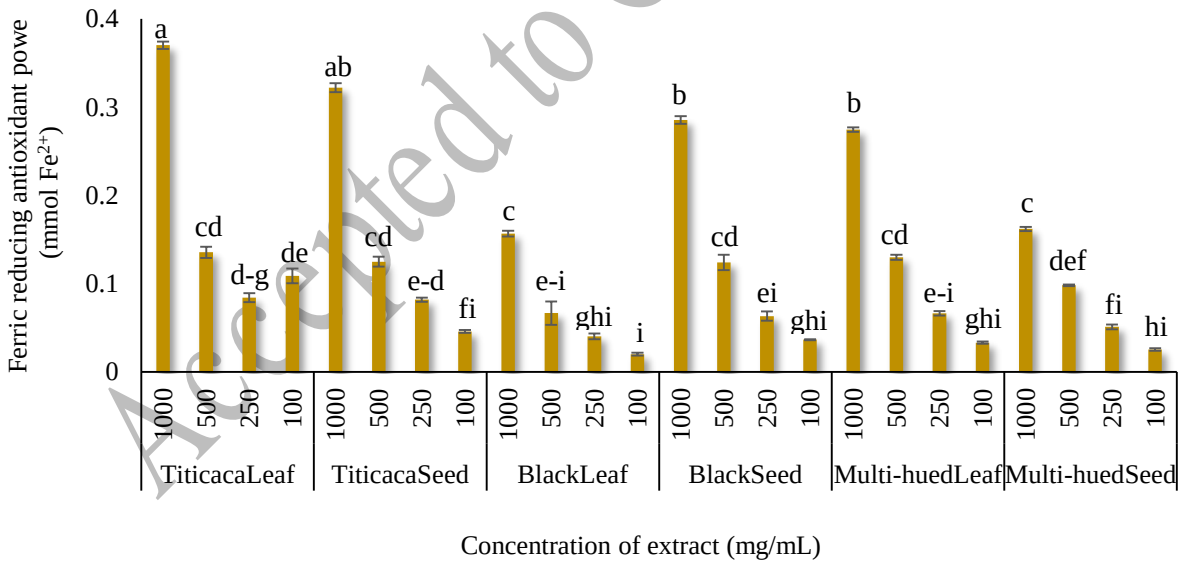

Fig. 7 Ferric reducing antioxidant power of different concentrations of leaf and seed extracts of the studied Quinoa cultivars The columns with a letter in common are not significantly different at P-value≤ 0.05

Studies indicated a correlation between the phenolic content and the FRAP, so that a higher phenolic content of the plant sample increases its FRAP [42]. The present study has also confirmed this correlation, considering the high phenolic content of Titicaca cultivar leaf and seed extract at 1000 mg/mL (Figure 3) compared to other plant samples of the studied cultivars. However, an investigation of the phenolic content of different samples of

the studied cultivars showed that the phenolic content and the FRAP decreased with the concentration of the extract (Figure 7). Many other compounds also play a role in determining the antioxidant power of the plant, such as non-enzymatic antioxidants (e.g., ascorbic acid, alpha-tocopherol, glutathione, carotenoids, proline, phenolic compounds, flavonoids, anthocyanins) and enzymatic antioxidants (e.g., superoxidase, catalase, and ascorbate-glutathione cycle enzymes).

On the other hand, different FRAP of Quinoa seeds were reported for different cultivars [43, 44]. Stikić *et al*. [30] stated that the reducing power of the seed extract obtained from the Titicaca variety (1.07) was higher compared to that of the Puno variety (0.82). These studies, confirming higher FRAP of the Titicaca cultivar, also identified high amounts of phenolic, flavonoid, and antioxidant compounds in seed extracts of the Titicaca cultivar compared to those of the Puno cultivar. Furthermore, the number of phytochemical compounds of Quinoa cultivated in different regions was different, so that the crude extract of Quinoa seed grown in Japan had higher antioxidant effects (scavenging free radicals and iron-reducing ability) than Quinoa cultivars grown in South America [45].

Based on variance analysis of the survival data of the MCF7 breast cancer cell line, studied samples showed significant differences (*P-value<0.01*) in all three time periods and different concentrations of the extract regarding the simple and triple interaction effects of the cultivar, type of plant sample, and time (Table 2). However, the interaction effect of cultivar and time at 1000 mg/mL with the kind of plant sample and time at 500 and 1000 mg/mL was not significant (*P-value>0.05*).

Table 2 The survival rate of the MCF7 cell line under different concentrations of leaf and seed extracts of Titicaca and Black Quinoa cultivars

Source of variance	df	Mean square			
		100	250	500	1000
Cultivar (cv)	1	112.68 **	70.09 **	335.17 **	778.44 **
Sample type (St)	1	169.43 **	246.50 **	97.74 **	404.27 **
Time (t)	2	19.72 **	34.89 **	65.66 **	448.71 **
cv*St	1	18.82 **	30.11 **	9.17 ns	60.22 **
cv*t	2	30.26 **	50.73 **	16.78 **	22.90 ns
St*t	2	55.29 **	19.59 **	6.63 ns	37.27 ns
cv*St*t	2	52.31 **	44.38 **	62.87 **	6.39 **
Error	24	2.21	0.79	2.55	6.88
Standard Deviation		4.37	4.37	4.35	4.80
R^2		0.88	0.88	0.96	0.89

ns and ** indicate not significant and significant difference at the 5% and 1%, respectively.

The lowest and highest survival percentages of MCF7 breast cancer cells were observed at 1000 mg/mL of Black cultivar leaf extract in 72 hours (13.92%) and 100 mg/mL of Titicaca seed extract in 24 hours (48.49%), respectively. Regardless of the extract concentration, the results showed a significant superiority (*P-value*<0.01) of leaf extract (35.26%) in comparison with seed extract (40.07%), Black cultivar (34.91%) in comparison with Titicaca cultivar (40.43%), and the time of 72 hours compared to 24 and 48 hours (Table 3 and Figure 8). The higher number of total flavonoids in the Black cultivar might lead to increased inhibition of cancer cells because the polysaccharides in Quinoa had a cytotoxic effect on the MCF7 breast cancer cell line, and no proliferation inhibition was observed on normal cells [16]. Phenolic compounds in Quinoa can prevent the proliferation of the MCF7 cell line in a concentration-dependent manner. Black Quinoa had a stronger inhibitory effect than red and white Quinoa [21]. Moreover, red and black Quinoa scavenged the proliferation of the MCF7 cell line by reducing the production and accumulation of nitrite, showing more effective anti-inflammatory and antitumor effects [33]. In line with the present result regarding the superiority of the toxicity effect of the leaf extract compared to that of the seed, Gawlik-Dziki *et al*. [46] reported the antitumor activity of phenolic extracts of Quinoa leaves by scavenging the proliferation of rat prostate cancer cells between 0.186 and 1.86 mg/mL.

Table 3 Comparison of the average toxicity effect of different concentrations of leaf and seed extracts of Titicaca and Black Quinoa cultivars on MCF7 cell line in 24 to 72 hours

Sample type	Cultivar	Time	T			
			1000	500	250	100
Leaf	Titicaca	24	41.05 a±2.40	41.72 abc±0.42	43.15 b±0.29	44.57 b±0.23
		48	36.09 b±0.46	40.57 bc±0.11	41.51 c±0.13	43.94 bc±0.25
		72	24.28 e±1.56	33.22 ef±0.97	34.29 e±1.58	38.67 e±0.47
	Black	24	27.48 de±0.25	30.57 f±0.78	31.47 f±0.25	32.76 f±0.61
		48	24.36 e±5.50	34.85 e±0.19	35.71e±0.04	37.99 e±0.15
		72	13.92 f±0.59	34.81 e±4.64	37.91 d±0.53	41.47 cd±2.88
Seed	Titicaca	24	42.27 a±0.23	44.23 a±1.44	45.42 a±1.95	48.49 a±0.08
		48	40.99 a±0.62	42.89 ab±0.21	43.78 b±0.30	44.40 b±0.17
		72	30.51 cd±1.34	41.30 bc±0.71	39.96 c±0.51	42.96 bc±1.17
	Black	24	33.10 bc±0.47	37.71 d±2.03	45.56 a±0.62	47.34 a±0.26
		48	31.97 bcd±6.39	36.95 cd±0.53	40.58 c±0.31	42.42 bcd±0.33
		72	28.55 cde±0.46	30.43 f±0.57	40.14 c±1.41	39.82 de±3.99

The numbers with a common letter are not significantly different at a *P-value* ≤ *0.05*.

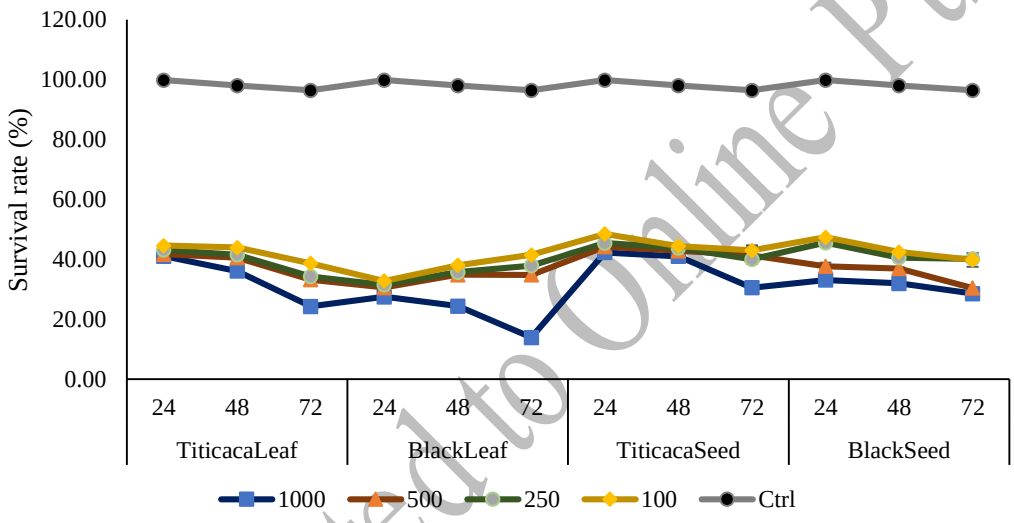

Fig. 8 Survival percentage of MCF7 cell line by MTT method of different concentrations of leaf and seed extracts of Titicaca and Black Quinoa cultivars in 24 to 72 hours

CONCLUSION

Based on the results, the total phenol and flavonoid content in the Titicaca and Black cultivars seed samples showed the highest values. The highest scavenging properties of DPPH free radicals and reducing iron elements were observed in the leaf samples of Black and Titicaca cultivars, respectively. At the same time, the lowest IC_{50} value was seen in the leaf sample of the Black cultivar. The phenolic, flavonoid, and antioxidant content increased with the increase of the extract concentration up to 1000 mg/mL in the leaf and seed samples. The highest value (*P-value*<0.05) was observed at 1000 mg/mL in all studied characteristics. Accordingly, the lowest survival percentage of the MCF7 breast cancer cell line was observed at the same concentration and using the Black leaf extract in 72 hours. Regardless of the extract concentration, the results showed significant superiority of leaf extract in comparison with seed extract, Black cultivar in comparison with Titicaca cultivar, and 72 hours compared to the periods of 24 and 48 hours (*P-value*<0.01). Future researchers should compare the present study findings with the cytotoxic effects of Black cultivar leaf and seed extracts on normal cells. In the case of confirming the results, clinical trials should be conducted to determine the best time and method of consuming Black Quinoa for treating or preventing breast cancer.

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FIGURE CAPTIONS

Fig. 1 A: Cultivation of studied cultivars in Yazd University research field, **B**: Labeling of randomly selected plants to measure the studied characteristics, **C**: Extraction of plant samples by Soxhlet method, and **D**: Preparation of samples to measure DPPH free radical inhibitory activity

Fig. 2 A: Flask containing MCF7 cell line studied in the Elizarider device and **B**: MCF7 cell line under the microscope
Fig. 3 Total phenolic content of different concentrations of leaf and seed extractions regarding the studied quinoa cultivars
The columns with a letter in common are not significantly different at P-value≤ 0.05
Fig. 4 Total flavonoid content of different concentrations of leaf and seed extractions of the studied quinoa cultivars
The columns with a letter in common are not significantly different at P-value≤ 0.05
Fig. 5 DPPH free radical scavenging of different concentrations of leaf and seed extractions of the studied quinoa cultivars
The columns with a letter in common are not significantly different at P-value≤ 0.05
Fig. 6 DPPH IC_{50} (mg/mL) of leaf and seed extracts of studied Quinoa cultivars
Fig. 7 Ferric reducing antioxidant power of different concentrations of leaf and seed extracts of the studied quinoa cultivars
The columns with a letter in common are not significantly different at P-value≤ 0.05
Fig. 8 Survival percentage of MCF7 cell line by MTT method of different concentrations of leaf and seed extracts of Titicaca and Black Quinoa cultivars in 24 to 72 hours