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Chemical, Antioxidant, Total Phenolic and Flavonoid Components and Antimicrobial Effects of Different Species of Quinoa Seeds

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QUINOA is considered as one of the newly emerged seed with high nutritional and biological aspects. The present research was done to study the biological activities of quinoa seeds. Quinoa seeds (cultivars; Giza1, Red Carina and Sajama) were obtained and their chemical properties were analyzed. Ethanoic extract was prepared and its total phenolic components (TPC), total flavonoid components (TFC), antioxidant and antimicrobial effects were studied. Saponin content was detected using the standard method. The highest contents of moisture, carbohydrate, protein, fat, fiber, ash and iron were found in cultivar Sajama (7.23±0.61%), Giza1 (63.45±4.25%), Red Carina (18.11±1.62%), Sajama (10.19±0.98%), Red Carina (5.75±0.51%), Red Carina (5.98±0.46%) and cultivar Giza1 (26.80±2.51), respectively. The mean concentrations of saponin in cultivar Giza1 had the highest concentration of saponin (4.25±0.23 mg/g), while cultivar Sajama had the lowest (1.84±0.12 mg/g). Cultivar Giza1 had the highest TPC, TFC and antioxidant effects. Cultivar Giza1 extract had the highest antibacterial effects. The minimum inhibitory concentrations of cultivar Giza1 (or Misr1) against *L. monocytogenes* and *E. coli* bacteria were 10 and 5 mg/ml, respectively, while those of *C. quinoa Sajama* against *L. monocytogenes* and *E. coli* were 80 and 40 mg/ml, respectively. The quinoa seeds are an appropriate alternative candidate of nutrients with high phenolic and flavonoid components and antimicrobial and antioxidant activities. High protein, carbohydrate, ash, fiber, iron, total phenol and flavonoid contents, low saponin concentration and considerable antibacterial and antioxidant effects of the quinoa seeds and particularly *C. quinoa Giza1* make them suitable as alternative functional sources of nutrients with high biological effects for human nutrition.

Keywords: Quinoa seed, Chemical properties, Antioxidant properties, Antimicrobial properties.

Introduction

Traditional medicine has continued as the major reasonable and simply available source of treatment in the primary health care system. Natural products have played a substantial portion in treating and preventing human diseases and also as a favorable additive in foods [1].

Quinoa (*Chenopodium quinoa*) is one of the oldest food crops native to the Andean areas of South America. Quinoa belongs to the *Amaranthaceae* family, *Chenopodiaceae* subfamily and Chenopodium genus. It has a boost tolerance to stressful abiotic circumstances and unbelievable nutritional values [2-4]. Quinoa's ability to harvest protein complex seeds in an

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inappropriate ecological circumstances brands it significant for the change of upcoming farming schemes [3-5]. Quinoa seeds have been presented in Europe, North America, Africa and Asia with high yields [2-5]. Nutritionally, the seeds comprise boost levels of protein and a complex of balanced amino acids than the old-style types of mueslis. It harbors a complex of nutritional fiber and beneficial fats [2-6]. Furthermore, the quinoa seeds comprise sufficient levels of vitamins and minerals [2-6]. Otherwise, quinoa seeds are rich sources of minerals (calcium, magnesium, potassium, iron, copper and zinc), vitamins (thiamin, riboflavin, niacin, ascorbic acid, α-tocopherol and β-carotene) and essential amino acids [2-6]. Previous investigation displayed that the contents of some minerals, vitamins and amino acids of the quinoa seeds are entirely higher than wheat, rice and barely [6]. Moreover, it is a latent irritating basis of phenolic, flavonoid and carotenoid complexes harbored healthy feathers [2-6]. High contents of phenolic, flavonoid and carotenoid compounds in the quinoa seeds guarantees its high antioxidant and antimicrobial effects [2-6]. Recognized documents revealed the high antioxidant and antimicrobial effects of different quinoa seed extracts against several types of bacteria [7, 8]. Quinoa collected in Asian countries has been classified into 17 races. *Chenopodium quinoa* cultivar Giza1, *Chenopodium quinoa* cultivar Red Carina and *Chenopodium quinoa* cultivar Sajama are the most frequently cultivated species of quinoa seeds in Iran [9].

Quinoa is also classified as 'sweet' or 'bitter' according to its saponin content. Although certain sweet diversities have been industrialized, some of the quinoa grown in Iran is of the bitter variety which poses a problem for its incorporation into food products [9]. Saponins are a big class of glycosides extant in all portions of herb with antibiotic, fungicidal, insecticidal, and therapeutic possessions [10]. Saponins are donated to the plant's defense against pests and pathogens. Ended 20 diverse kinds of saponin have been defined in dissimilar shares of plant (fruits, flowers, seed furs and seeds) [10, 11]. They are mostly have a forcefully bitter flavor which decreases the organoleptic properties and the delectableness of their sources. Accordingly, it is significant to evaluate their concentrations in different species of quinoa [10, 11].

Quinoa seeds have high nutritional, antioxidant and antimicrobial effects. However, there were no beforehand published data about the chemical properties and antioxidant and antimicrobial effects of these species of quinoa seeds. Therefore, the present investigation was done to study the content of protein, carbohydrate, ash, fat, fiber, moisture, iron, total phenolic components (TPC), total flavonoid components (TFC), saponin content and antimicrobial and antioxidant effects of cultivar Giza1, Red Carina and Sajama seeds.

Materials and Methods

Ethical consideration

The study was accepted by the Moral Assembly of Research of the Faculty of Veterinary Medicine, Shahrekord Branch, Shahrekord, Iran (Consent Ref Number 940433740). Corroboration of this research project and the authorizations related to sampling process were approved by the Prof. Amir Shakerian, Prof. Ebrahim Rahimi and Dr. Mahmoud Bagheri.

Chemical reagents

Folin-Ciocalteau reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Butylated hydroxytoluene (BHT), formic acid, gallic acid, p-hydroxybenzoic acid, vanillic acid, p-coumaric acid, ferulic acid, quercetin and kaempferol were supplied by Sigma-Aldrich Chemical Company (St. Louis, USA). Ethanol (99% purity), methanol (95% purity), sodium carbonate, aluminum chloride, sodium nitrite, sodium hydroxide, Muller Hinton agar, MacConkey agar and listeria selective agar media were supplied by Merck company (Darmstadt, Germany). Amoxicillin (100 µg/disk) disk was prepared from Oxoid company (Oxoid, UK).

Plant material

Quinoa seeds (cultivars; Giza1, Red Carina and Sajama) were obtained from the Seed and Plant Improvement Institute (SPII), Karaj, Iran. Plants were cultured on May 2017 and then harvested on September 2017. Seeds were cleaned and stored in polyethylene containers at room temperature until use. Before extraction, the seeds were crushed using a laboratory grinder (Yellow line, A10, IKA-Werke, Staufen, Germany) and sifted (<0.5 mm particle size). The fine powder was packed and stored at room temperature in a dry and dark place until use.

Chemical properties of quinoa seeds

Protein content of sample (about 0.3 g) was analyzed by micro-Kjeldahl method (digester: F30100184, SN: 111051, VELP Scientifica;

distiller: F30100191, SN: 111526, Europe) using urea as control. Protein (%) = N (%) × 6.25 [12]. Moisture content was analyzed by drying in oven (Model: 101-1A, Tianjin Taisite Instrument Co. Ltd.). Sample (about 3.0g) was dried at 100˚C for about 6 h [12]. Fat content was measured by the Soxhlet extraction (Model: EV 16, SN: 4002824, Germany) of sample (about 2.0 g) using petroleum ether as a solvent [12]. The content of fiber was determined by taking about 3.0 g sample as portion of carbohydrate that resisted sulfuric acid (1.25%) and NaOH (1.25%) digestion, followed by sieving (75 µm), washing, drying and ignition to subtract ash from fiber [12]. Total ash content was determined by ashing about 3.0 g sample in a muffle furnace (Model: MF120, SN: 04-1524, Ankara Turkey) at 550˚C until ashing complete (over 12 h) [12]. Iron content was analyzed after digestion of sample (about 2.0 g) by measuring absorbance of Fe2+ -1, 10-phenanthroline red complex color at 510 nm using UV-VIS spectrophotometer. The iron level was estimated from standard calibration curve (0.0 - 10.0 µg Fe/ml) prepared from analytical grade iron wire [13]. Carbohydrate content was calculated based on the method defined by Merrill and Kunerth [14]. All chemical properties were presented as percent. Iron content was presented as part per million (ppm).

Preparation of extract

Two grams of quinoa seeds were homogenized with 20 mL of 80% ethanol. The mixture was kept in agitation for 30 min at 160 rpm in an orbital shaker. Then, the homogenate was centrifuged for 10 min at 11000 rpm (Eppendorf Centrifuge 5804, Hamburg, Germany) and the supernatant was removed. The residue was extracted once again at the same conditions. Then, both supernatants were pooled, filtered (0.45 µm) and stored at -18°C for further analysis.

Analysis of total phenolic content (TPC)

TPC of three different quinoa seed extracts was determined using Folin-Ciocalteau reagent according to the method described previously [15]. The extracts were diluted and mixed with Folin-Ciocalteau reagent (2 N) and 20% sodium carbonate solution. The mixture was incubated in the dark place for 2 h. After incubation, the absorbance of the mixture was measured at 765 nm using an UVmini 1240 spectrophotometer (Shimadzu, France). The results were presented as equivalent of Gallic acid (GAE) in mg per 100 g quinoa seeds in dry weight basis (dw).

Analysis of total flavonoid content (TFC)

TFC was analyzed by the aluminum chloride colorimetric technique according to method described previously [16]. Fleetingly, 0.25 mL aliquot of the extract was mixed with 2 mL of distilled water and 0.15 mL of 5% sodium nitrite solution in a test tube. After 5 min, 0.15 mL of 10% aluminum chloride solution was added. At 6 min, 1 mL sodium hydroxide solution (1M) was added to the mixture. Unswervingly, the solution was diluted with 1.2 mL of distilled water and carefully mixed. Absorbance of the final mixture was determined at 510 nm using the spectrophotometer (Shimadzu, France). Total flavonoid content was presented as equivalent of quercetin (QE) in mg per 100 g quinoa seeds in dry weight basis.

Analysis of antioxidant effects

Antioxidant activity of quinoa seed extracts was evaluated by measurement of 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity [17]. Aliquots (50 µL) of extracts were added to 1,950 µL of a methanolic solution (40 µM) of DPPH radicals. After agitation, the mixture was incubated in the dark place for 30 min and the absorbance was measured at 517 nm using the spectrophotometer (Shimadzu, France). The antioxidant activity was presented as percentage of DPPH radical scavenging calculated based on the following equation [18]:

% DPPH radical scavenging =

$$[(A_{C(30)}\text{-}A_{S(30)}/A_{C(30)})] \times 100$$

Where $A_{C(30)}$ corresponds to absorbance of DPPH radical + methanol at t=30 min and $A_{S(30)}$ to absorbance of DPPH radical + sample at t=30 min.

Saponin detection

Saponins used to calibrate the method were extracted from commercially available bitter quinoa representing a mixture of varieties. Whole seeds (30 g) were first defatted by Soxhlet extraction with diethyl ether for 6 h. Saponins were extracted with 250 ml of 80% methanol under reflux for 4 h, and the extract was filtered and then evaporated to dryness. The dried extract was dissolved in *n*-butanol/ethanol/water (1 :1:1 v) using 5 ml of solvent for each 1 g of extract, and applied to a column of aluminum oxide of 125 ml bed volume to eliminate the pigments extracted along with the saponins. Saponins were eluted from the column with 250 ml of the similar solvent and the eluate was evaporated to dryness.

This crude saponin extract was stored desiccated over silica gel at 25-27 °C. Instructions were completed according to the method presented by Kozio (1991) [19].

Bacterial strains and growth conditions

Listeria monocytogenes (ATCC 15313) and *Escherichia coli* (EHEC: NCCP 13721) were obtained from the Iranian Industrial and Scientific Research Center (Tehran, Iran). Stock cultures were maintained at -80 °C in broth containing 20% glycerol (Sigma-Aldrich, UK). Simple tryptic soy broth (TSB, Merck, Germany) and TSB with 0.6% yeast extract (Oxoid, Hampshire, UK) were used to culture the *E. coli* and *L. monocytogenes*, respectively. For each experiment, the stock culture of each bacterium was thawed at room temperature. Then, 0.01 mL of thawed stock culture of the bacterium was inoculated into a 25 mL Erlenmeyer flask containing 10 mL of nutrient broth (NB, Merck, Germany), wrapped with a silicone cap and incubated aerobically at 37 °C for 24 h.

Analysis of antimicrobial effects of quinoa seeds against Escherichia coli and Listeria monocytogenes

Bacterial inoculates were growth on Nutrient broth (NB) (Merck, Frankfurt, Germany) agar at 37 °C for 24 h; Colonies were added in sterile 0.9% saline and adjusted to 0.5 McFarland, which is equivalent to 10^8 colony-forming units per mL (10^8 CFU/mL). Antimicrobial effects of the quinoa seed extracts were tested using the simple disk diffusion method [20]. The bacterial suspension was adjusted to a density of bacterial cells of 1.0 $\times 10^8$ CFU/mL (or 0.5 McFarland turbidity units). Sterile paper discs (9 mm in diameter and 250 g m-2) were impregnated with 25 µL of pure extract and placed on plates inoculated with 10^8 suspensions of each culture, which were then incubated at 37 °C for 18-24 h. The diameter of growth inhibition zones, including the disc diameter, was measured in millimeters [20]. Tests were performed in quadruplicate. Antimicrobial susceptibility of bacterial strains was also studied against certain antibiotic disks. For this purpose, the dimeter of the growth inhibition zone for each treatments of extract were analyzed. Then, the diameter of the inhibition zones of those that had the highest antimicrobial activities (the highest diameter of the zone of inhibition) were compared with different antibiotic agents. For this purpose, amoxicillin (25 µg/disk), gentamicin (10 µg/disk), cefexime (5 µg/disk), and tetracycline (30 µg/disk) antibiotic disks were used (Oxoid, Wade Road Basingstoke Hampshire RG24 8PW, UK). Analyses was done according to the method descried by the Clinical Laboratory Standard Institute (CLSI, 950 West Valley Road, Suite 2500 Wayne, PA 19087 USA) [21].

Minimum Inhibitory Concentrations (MIC)

The minimum inhibitory concentrations (MICs) were determined using 96-well microtitre plates. Wells of 1 to 6 were considered for different concentrations of the quinoa seed extracts (5, 10, 20, 40 and 80 mg/mL). Row number 7 contained the Mullet Hinton Broth media (100 µL) (MHB, Merck, Frankfurt, Germany) and microbial suspension (100 µL) as positive control. Row number 8 contained the Mullet Hinton Broth media (200 µL) (MHB, Merck, Frankfurt, Germany) as negative control. Totally, 100 µL of related concentrations of each well were added into the wells of 1 to 6. In all wells (except row number 8), 100 µL of bacterial suspensions with turbidity equal to 1.5×10^7 bacteria/ml were added. Immediately after completion, the optical absorbance of the microplate wells was read using the microplate reader device (Model 680, Bio-Rad Laboratories Inc., Berkeley, CA, USA) at 630 nm. Then, the microplate was incubated for 24 h at 37 °C and its optical absorbance was read another time using the ELISA reader device. The MIC values were determined using the comparison of the optical absorption of each well before and after incubation period and also the ocular examination of the opacity. Therefore, the lowest dilution of the test substance without any opacity (in the wells of that concentration) was considered as the MIC [22].

Statistical analysis

Data were transferred into the Microsoft Excel software (Microsoft Corp., Redmond, WA, USA). Results were then analyzed using the SPSS 21.0 statistical software (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) test was used for study the presence of significant differences between data recovered from the current research.

Results

Chemical properties and saponin content of quinoa seeds

Table 1 represents the chemical properties of different quinoa seeds. The highest contents of moisture, carbohydrate, protein, fat, fiber, ash and iron were found in *C. quinoa sajama*

(7.23±0.61%), *C. quinoa Giza1* (63.45±4.25%), *C. quinoa red carina* (18.11±1.62%), *C. quinoa sajama* (10.19±0.98%), *C. quinoa red carina* (5.75±0.51%), *C. quinoa red carina* (5.98±0.46%) and *C. quinoa giza* (26.80±2.51), respectively. The mean concentrations of saponin in *C. quinoa giza*, *C. quinoa red carina* and *C. quinoa Sajama* were 4.25±0.23, 3.84±0.18 and 1.84±0.12 mg/g, respectively. There were no significant statistical differences for contents of moisture, carbohydrate and fiber between different quinoa seeds (P >0.05). Statistically significant differences were found for the contents of protein, fat, ash, iron and saponin between different quinoa seeds (P <0.05).

Phenolic and flavonoid contents and antioxidant activities

Table 2 represents the TPC and TFC and DPPH radical scavenging activity of different quinoa seeds. We found that quinoa seed extracts had considerable amount of TPC, TFC and radical scavenging activity. The highest contents of TPC and TFC and DPPH radical scavenging activity were found in *C. quinoa giza* (38.24±2.45 mg GAE per 100 g dw), *C. quinoa giza* (22.10±1.82 mg quercetin per 100 g dw) and *C. quinoa giza* (25.33±2.11%), respectively. Statistically significant differences were found for the contents of TPC and TFC and DPPH radical scavenging activity between different quinoa seeds (P <0.05).

Antibacterial effects of quinoa seeds

Table 3 describes the diameter of growth inhibition zone of different quinoa seeds and several antibiotic agents against *L. monocytogenes* and *E. coli* bacteria. The highest diameter of the growth inhibition zone of *L. monocytogenes* and *E. coli* bacteria was found for *C. quinoa giza* extract (12.42±0.41 mm), respectively. Statistically significant differences were found for the diameter of the growth inhibition zone of *L. monocytogenes* and *E. coli* bacteria between *C. quinoa giza* and two other species of quinoa seeds (P <0.05). Cefexime (15.71±1.35 mm) had the higher diameter of the growth inhibition zones of *L. monocytogenes* and *E. coli* (12.42±0.41) (P <0.05) and (10.24±0.89 mm) respectively compared to *C. quinoa giza*1 extract. Statistically significant difference was found for the diameter of the growth inhibition zone of the *E. coli* between *C. quinoa giza* extract and other quinoa seeds and antibiotic agents (P <0.05).

MICs of quinoa seeds

Table 4 represents the MICs of different quinoa seed extracts against *L. monocytogenes* and *E. coli* bacteria. The MICs of *C. quinoa giza* against *L. monocytogenes* and *E. coli* bacteria were 10 and 5 mg/ml, respectively which was lower than other two species of quinoa seed. The highest MIC values of *L. monocytogenes* and *E. coli* were found for C. *quinoa sajama* (80 and 40 mg/ml, respectively).

Discussion

Extracts from plants and seeds contain numerous diverse components which can provoke additive and/or synergic effects on biological systems; thus, their uses in traditional medicine and also food science are being authorized [23, 24]. Quinoa seeds have long been considered possible dietary supplement rich in phenolic, flavonoid, antioxidant and antimicrobial compounds [23, 24]. Additionally, it has appropriate conditions to use as a valuable supplement for production functional food [23, 24]. In keeping with this, little information is available concerning the nutritional, antioxidant and antimicrobial potential of quinoa seeds.

The present investigation was done to study the contents of protein, carbohydrate, ash, fat, fiber, moisture, iron, TPC, TFC and antimicrobial and antioxidant effects of *C. quinoa giza*, *C. quinoa red carina* and *C. quinoa sajama* seeds. We found diverse chemical, TPC, TFC, antioxidant and antimicrobial properties for three different species. Chemical properties obtained in our survey were similar with those reported by Meneguetti et al. (2011) [5]. Gordillo-Bastidas et al. (2016) [25] and Park et al. (2017) [7] (Korea). Quinoa seeds cultivated in Iran (the current research) contained a higher amount of protein (18.11%), fat (10.19%), carbohydrate (63.45%) and ash (5.98%) than previously published data [26-28]. Diversely, the moisture content (7.23%) was lower than those of previous investigations [26-28]. Iron content of our studied quinoa seeds (26.80 ppm) was lower than that of Spain (261.2-333.8 ppm) [29], while was higher than that of Serbia [30] (18 ppm). Additionally, the content of fiber of our studied quinoa seeds (5.75%) was higher than Spain (2.5%) [29] and Serbia (1.7%) [30]. It must be renowned that the quinoa seed's protein content in the current research (18.11%) was higher than some other more broadly consumed ounces, for instance wheat (*Triticum aestivum* L.) (14.50-16.70%), maize (*Zea mays* L.) (8.9-10.2%), oat (*Avena sativa* L.) (11.50%), rice (*Oryza sativa* L.) (7.50-7.90%) and barley (*Hordeum vulgare* L.) (10.8-11.8%) [2, 23, 31]. Furthermore, studied chemical properties were higher or in the rages of previously studied feathers of wheat, maize, and rice [32]. Consequently, quinoa can address as a substitute source of nutrients and especially protein by people with dietary protein deficiency.

TABLE 1. Chemical properties of different quinoa seeds.

Types of seeds	Chemical properties*							
	Moisture (%)	Carbohydrate (%)	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	Iron (ppm)	Saponin (mg/g)
C. quinoa giza	6.93±0.66^{a**}	63.45±4.25^{a}	17.67±1.65^{a}	6.60±0.54^{b}	5.00±0.43^{a}	5.16±0.38^{a}	26.80±2.51^{a}	4.25±0.23^{a}
C. quinoa red carina	6.85±0.613^{a}	62.71±3.71^{a}	18.11±1.62^{a}	2.55±0.21^{c}	5.75±0.51^{a}	5.98±0.46^{a}	21.65±1.88^{ab}	3.84±0.18^{a}
C. quinoa sajama	7.23±0.61^{a}	60.01±5.12^{a}	15.50±1.39^{b}	10.19±0.98^{a}	3.44±0.27^{a}	3.02±0.28^{b}	24.85±2.18^{a}	1.84±0.12^{b}

*All results were presented as mean of triplicate tests with standard deviation.
**Unlike minor leathers in each column illustrate noteworthy numerical alteration (P <0.05).

TABLE 2. Entire phenolic and total flavonoid profiles and DPPH radical scavenging capacity of different quinoa seeds.

Types of seeds	TPC (mg GAE per 100 g dw)*	TFC (mg quercetin per 100 g dw)	DPPH radical scavenging effects (%)
C. quinoa giza	38.24±2.45^{a**}	22.10±1.82^{a}	25.33±2.11^{a}
C. quinoa red carina	21.30±1.24^{b}	14.86±1.45^{b}	20.76±1.34^{b}
C. quinoa sajama	17.58±1.12^{b}	6.28±3.61^{c}	15.25±1.12^{c}

*All results were presented as mean of triplicate tests with standard deviation.
**Unlike minor leathers in each column illustrate noteworthy numerical alteration (P <0.05).

TABLE 3. Comparison of the diameter of growth inhibition zone of different quinoa seeds and several antibiotic agents against *L. monocytogenes* and *E. coli* bacteria.

Types of seeds	Diameter of the growth inhibition zone (mm)*	
	L. monocytogenes	*E. coli*
C. quinoa giza	12.42±0.41^{b**}	15.01±0.72^{a}
C. quinoa red carina	7.23±0.18^{c}	9.85±0.63^{b}
C. quinoa sajama	4.01±0.12^{d}	7.14±0.68^{c}
Amoxicillin	10.25±1.14^{b}	9.12±0.84^{b}
Gentamicin	4.46±0.31^{d}	5.23±0.48^{c}
Cefexime	15.71±1.35^{a}	10.24±0.89^{b}
Tetracycline	6.27±0.51^{c}	6.27±0.57^{c}

*All results were presented as mean of triplicate tests with standard deviation.
**Unlike minor leathers in each column illustrate noteworthy numerical alteration (P <0.05).

TABLE 4. Minimum inhibitory concentrations of different quinoa seeds against *L. monocytogenes* and *E. coli* bacteria.

Types of seeds	Minimum inhibitory concentrations (mg/ml)	
	L. monocytogenes	*E. coli*
C. quinoa giza	10	5
C. quinoa red carina	20	10
C. quinoa sajama	80	40

Results of the present investigation was also showed that the different quinoa seeds contain low concentrations of saponin (1.84-4.25 mg/g). Low saponin content of quinoa seeds will guaranty their sweet falvour. *C. quinoa giza* had the highest concentration of saponin (4.25±0.23 mg/g), while *C. quinoa Sajama* had the lowest (1.84±0.12 mg/g). Contents of the saponins in *C. quinoa red carina* was 3.84±0.18 mg/g. Attendance of erratic concentrations of saponins was described numerous comestible seeds and frequently a restraint for a straight application since bitter taste and unidentified complications. The saponin quantity present in the quinoa seeds be contingent on genotype: it is advanced in varieties with bitter-flavour, or low-saponins, diversities [33]. Developmental phase of the crop is additional factor on the saponin content, being low throughout branching and boost throughout flowering [34]. Drought caused 45% decrease on buildup of sapogenins in quinoa [11], whereas salinity has the opposite effect [35]. Though, a important upsurge of saponins and supplementary components of seed has been described in an irrigated parts as opposite to a cold climate [36, 37]. Consequently, it has been recommend that supplementary researches must be led to clarify how the environmental and the genotypic factors inspiration the saponin content of seed [38]. Lower concentrations of saponins were reported in the quinoa seeds cultivated in Italy (0.1-1.80 mg/g) [39], USA (0.30 mg/g) [40] and Pakistan (0.38- 0.63 mg/g) [41], while higher saponin contents were reported from Germany (0.1-3.50 mg/g) [42] and Italy (5.6 to 7.5 mg/g) [11].

Since saponins, because of their intrusion with delectableness of quinoa and digestibility, have to be detached before consumption, this seems to be a promising possessions of *C. quinoa Sajama* compared to additional cultivars examined. Other wise, sweet genotypes frequently display low pest-resistance. Certainly, saponins were described to apply a robust insecticidal or defensive action toward a comprehensive variety of herbivores (e.g., birds) insects, and even microbial infections. Thus, its low concentration will increase the sensitivity of the plant to environmental factors. Generally, saponin contents of tested bitter cultivars can be stared as low in comparison to additional researches reporting ranges from 4.8 to 11.4 mg/g [11, 35]. These inconsistencies are perhaps accredited to accompanied method of experiment.

Polyphenols are subordinate bioactive metabolites that are extensively present in typically consumed foods by plant origins. The two chief kinds of polyphenols are phenolic acids and flavonoids which perform as influential antioxidant agents [26, 43-45].

The attitude of the antioxidant effects is the obtainability of electrons to counteract any so-called free radicals. Contents of TPC, TFC and DPP radical scavenging of studied quinoa seeds had ranges of 67.58±5.12 to 95.24±8.45 mg GAE per 100 g dw, 6.28±3.61 to 22.10±1.82 mg quercetin per 100 g dw and 57.49±4.72 to 81.33±6.51%, respectively. High antioxidant effect of the quinoa seeds is mostly because the boost gratified of TFC and TFC. Quinoa presents at least 23 phenolic compounds [26, 43-45]. Previous studies revealed that the TPC of white, red and black quinoa seeds were 466.99, 634.66 and 682.05 mg/kg quinoa, respectively [26, 43-45]. The most abundant phenolic components in the quinoa seed extract are quercetin and ferulic acid [26, 43-45]. Quinoa contains advanced amounts of phenols than mueslis, for instance rice, wheat, barley, millet, and buckwheat [26, 43-45]. Alvarez-Jubete et al. (2010) [46], Graf et al. (2015) [47], Abderrahim et al. (2015) [48], Pasko et al. (2009) [49] and Gawlik-Dziki et al. (2013) [50] reported higher antioxidant, TPC and TFC (especially kaempferol glycosides and quercetin) of the quinoa seeds than other types of cereals and pseudocereals. They recommended that quinoa may signify a significant source of inhibition of free radicals and can use as a functional food because of high content of TPC, TFC and anthocyanins. Nsimba et al. (2008) [51] described that the TPC and DPPH radical scavenging activity of different quinoa seed extracts had ranges of 94.3±3.0 to 148.0±1.9 mg GAE per 100 g dw and 59.2±1.4 to 85.6±0.9%, respectively which was higher and lower than our findings. Contents of the TPC, TFC and antioxidant activities of quinoa seed cultivated in Argentina [52] were lower (39.29±0.92 mg GAE per 100 g dw), lower (11.06±0.42 mg quercetin per 100 g dw) and lower (13.61±1.10%) than our findings, respectively. Chacaliaza-Rodríguez et al. (2016) [53] reported that the TPC and TFC of two quinoa cultivars of Salsado and altiplane cultivated in Peru were 10.55±0.35 and 10.72±0.27 mg GAE per 100 g dw and 8.69±0.49 and 9.14±0.42 mg quercetin per 100 g dw, respectively which were lower than our findings. The difference in the TPC, TFC and antioxidant activities of three different quinoa seeds might be elucidated by the alteration in the environmental circumstances, agrotechnical processes and genetic contextual. Additionally, a comparison of the fallouts of diverse researches can be problematic because of the erraticism in the investigational circumstances amongst the applied techniques.

Antimicrobial effects of the quinoa seed extract showed that the bacterium that showed the highest inhibition zone was *E. coli* (10.01±0.72 mm). Though, the inhibition zone was lower than 10 mm and could be understood as the plant essential oil have no sensitivity toward the microorganisms. Particularly, slight inhibition aptitude was seen toward *L. monocytogenes*, and there was noteworthy variance in the antimicrobial effect among the quinoa seeds. *C. quinoa giza* extract had the higher antimicrobial effects against *E. coli* and *L. monocytogenes* than other two species of quinoa seeds (P <0.05). Our findings are slightly similar with those of Miranda et al. (2014) [8], in which six various sources of quinoa seeds had antimicrobial activity toward *E. coli* (8.29 to 14.79 mm) and *Staphylococcus aureus* (*S. aureus*) (8.53to15.03 mm). Park et al. (2017) [7] reported that the quinoa seeds cultivated in Korea, USA and Peru didn't have any antimicrobial effects against *L. monocytogenes*, While the dimeter of the growth inhibition zone of quinoa seeds cultivated in Korea, USA and Peru against *E. coli* were 7.04±0.11 mm, 7.35±0.35 mm and 7.35±0.25 mm, respectively which was lower than our recorded data. Antibacterial effect of the quinoa seeds against *E. coli* was also reported by Pagno et al. (2016) [54]. Miranda et al. (2014) [8] reported that the growth inhibition zones of the Ancovinto, Cancosa, Cahuil, Faro, Regalona, Villarrica ecotypes of the quinoa seeds against *E. coli* were 12.80±0.72 mm, 14.79±0.21 mm, 9.85±0.50 mm, 9.54±0.67 mm, 8.29±0.22 mm and 9.35±0.48 mm, respectively. Alteration in the activity of quinoa among researches could be incompletely clarified by differences in bioactive agents of extract and sensitivity of strains.

Such researches established that bacterial cell wall components, including peptidoglycans, teichuronic and teichoic acids and lipopolysaccharides (LPS) are accountable for the antibacterial activities of quinoa seeds. Additionally, presence of TPC is another factor which guarantees the antimicrobial effects of the quinoa seeds. Khoobchandani et al. (2010) [55] stated that most of the higher sensitivity of Gram-

positive bacteria toward antibiotics and essential oils. Hereafter the outstanding application of the seed extract with Gram-negative (*E. coli*), as well as Gram-positive (*L. monocytogenes*), was predominantly motivating.

Conclusion

To put it in a nutshell, we identified a considerable amount of TPC and TFC and antioxidant and antimicrobial effects for three different quinoa seeds cultivated in Iran. *C. quinoa giza* contains the highest levels of TPC, TFC and DPPH radical scavenging activity. Our data revealed that the contents of protein, fat, fiber and iron in studied quinoa seed samples were higher than other species of the quinoa and also some other cereals and pseudocereals. Furthermore, low concentrations of saponin found in almost all studied quinoa seeds guarantees their sweet flavor. Consequently, quinoa seeds could be applied as an substitute source of nutrients and particularly protein with boost phenolic and flavonoid components and antimicrobial and antioxidant activities by people with dietary nutrient deficiency. It has also the capacity of using as functional foods with considerable antimicrobial effects against *E. coli* and *L. monocytogenes*. However, additional studies are required to understand more information about the other nutritional and physical aspects of quinoa seeds.

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Conflict of Interest

The author has no conflict of interests to declare regarding the publication of this paper.

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