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NUTRITIONAL AND ANTINUTRITIONAL CHARACTERIZATION OF *CHENOPODIUM ALBUM* SEEDS: A NEGLECTED WILD SPECIES

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ABSTRACT

The nutritional and anti- nutritional characteristics of the seeds of *C. album* and *C. quinoa* were investigated using standard analytical methods. The results indicate that the seeds of both *C. album* and *C. quinoa* were an excellent source of protein, lipids, sugar, minerals and vitamins. The phytochemical screening of the seeds show the presence of alkaloids, amino acids, anthraquionones, flavonoids, gycosides, phytosterol, saponins, steroids, tannins and triterpenoids. On the other hand, various antinutrients like flavonoids, saponins, phytic acid, phenols and tannins were also present in appreciable amounts. The ethanolic seed extracts of *C. quinoa* were better antioxidants in comparison to *C. album*. Thus, the present analysis indicates that seeds of *Chenopodium album* have high nutritional value and as this crop is freely available and thrown as weed, it may be used as cereal without any cost and hence help in removing food scarcity, if used wisely. Therefore, it is recommended for future commercial cultivation and consumption.

KEYWORDS

C. album, Phenols, Tannins, Saponins, Phytic acid and Anti - oxidant activity.

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INTRODUCTION

The genus *Chenopodium* contains about 250 species of which about eight species are found in India. Chenopodium quinoa, commonly known as quinoa, is a well-known staple food of the Andean communities serving as an alternative food source in other regions[1,2]. Quinoa seeds are used as a potential crop for many animal feed formulations, for direct feeding, and as a human food[3] due to high-protein and a balanced amino-acid spectrum with high lysine (5.1–6.4%) and methionine (0.4–1.0%) contents[4,5]. The seeds are used in the same way as rice and

wheat and are ground into flour to prepare breads, cakes, and fermented drinks[6,7]. It belongs to the group of crops known as pseudo cereals that includes other domesticated chenopods, amaranths and buckwheat. The seed contains high-quality protein and is particularly rich in essential amino acids and its carbohydrate content has been reported to have a higher nutritional value than that of cereals such as corn, oats, wheat and rice[8,9]. Quinoa seeds are also rich in mineral nutrients (3.0%), and the K, Ca, Mg, P and Fe contents are much higher than those of conventional cereals[10-13]. In the last few decades, quinoa has been evaluated as a food with excellent nutritional characteristics by the National Research Council and the National Aeronautics and Space Administration (NASA)[14,15] and has been noted as a new foodstuff in the world. Thus, recently there has been growing interest in a number of countries (especially in Europe), initiating introduction and research work on quinoa[16].

Chenopodium album, which also belongs to the same genus, is a common weed in India. Although eaten as a leafy vegetable in many parts of the country, this plant has been considered only as a source of poultry feed[17]. *C. album* is the most widely distributed and is grown in the Himalayan region. The Himalayan grain Chenopod is comparable to Andean *quinoa* in nutrient composition and is much better than wheat, barley, maize and rice. Grain protein quality equals that of milk and contains high lysine (6 g /100 g protein), methionine (2.3 g/ 100 g protein) and cysteine (1.2 g /100 g protein)[18]. The grains are also used in local alcoholic preparations and out of the four domesticated species, *C. album* is the most widely distributed and is grown in the Himalayan region[18]. The crop is also suited to the mixed farming system, particularly the multiple cropping systems. Analysis of foliage of ten species of *Chenopodium* at the National Botanical Research Institute (NBRI), Luck now, revealed a wide range of variation for protein (26–64 g/ kg), carotene (78–190 mg/ kg), vitamin C (0.5–2.4 g/ kg), nitrate (2.6–5.0 g /kg) and oxalate (9–39 g/ kg) [19].

The plant is commonly available in India but day by day its popularity is decreasing due to lack of awareness about its unique medicinal properties. *Chenopodium album* is relatively inexpensive, easily and quickly cooked green leafy vegetable. One does not find much literature on seeds of *C. album* as it is generally discarded from the field as a weed[20]. Since, *C. album* and *C. quinoa belong to same genus and thus, it is assumed that the seeds of C. album* may possess similar nutritional characteristics as are well evident in *C. quinoa.* Data on the nutritional composition of the leaves of this plant is available[21-24] but data on nutritional composition of its seeds is limited. In this study, an attempt is made to determine the chemical composition of the seeds of this neglected plant so that it may be grown as a crop and provide nutritional support to human society and reduce food scarcity of poor people.

MATERIAL AND METHODS

Sample Collection

The seeds of *Chenopodium album* viz. A, B, C (cultivated and wild) were collected from different places in and around the Dayalbagh University campus, Agra (27°10'N, 78°05'E, and 169 m.s.l.; located in the north central part of India). The study area is about 10 km away from the industrial sector of the Agra city where due to agricultural practices vegetation predominates. The seeds of *C. album* A, B, C were collected in the month of March for three consecutive years 2010, 2011 and 2012 at the full maturity stage. The seeds of *C. album* B were cultivated in the botanical garden of Institute campus while two wild varieties were collected in duplicate from wheat (*C. album* A) and gram fields (*C. album* C) of Dayalbagh, Agra. The fields of Dayalbagh are situated about 5 km away from the Institute campus and consist of villages which are generally classified as rural and poor. However, treated seeds of *C. quinoa* were obtained from USA. Professor Anil Kumar Bhatnagar, Department of Botany, Dayalbagh University campus, authenticated the species. A voucher specimen was prepared and deposited in the herbarium of the Department of Botany (Pachauri Med. 2010/11). The seeds were allowed to air - dry in a shaded and well-ventilated place at ambient temperature (± 24°C) were ground into fine powder using mechanical grinder and then

were sieved to maintain uniform size for chemical and nutritive analysis.

Chemicals

Sigma grade (St. Louis, MO, USA) chemicals viz. 1, 1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, polyvinylpolypyrrolidone (PVPP), N-acetyl-DL-phenylalanine 2-naphthyl ester, trypsin, tetrazotized o-dianisidine, *N, N* dimethylformamide, N-acetyl-DL-phenylalanine 2-naphthyl ester, catechin, sodium phytate and quercetin were used. Folin - Ciocalteus's phenol reagent, sodium carbonate, metaphosphoric acid, 2, 6-Dichlorophenol indophenol dye, Ascorbic acid solution, β- carotene solution, $FeCl_3$ and $AlCl_3$ were from Merck Chemical Supplies (Damstadt, Germany). All the other chemicals used including the solvents, were of analytical grade.

Proximate analysis

Moisture, ash, protein, carbohydrates (reducing and total sugars) and lipids were analyzed by the methods described in[25]. Moisture was determined by drying oven method, by drying 5 g sample in an oven at 105° C for 3 hours. Ash content was determined by incineration of the samples (2 g) in a muffle furnace at 600° C for 6 hours until the ash turned white. Protein content of the seed samples was estimated colorimetrically by Lowry method for which the samples were extracted in phosphate buffer by centrifugation. Nelson- Somoygi's method was used for the determination of reducing sugar for which the seed samples were extracted with water. On the other hand, total sugar was obtained by hydrolyzing the seed samples with conc. HCl and then estimated by potassium ferricyanide method, spectophotometrically. Seeds were extracted with chloroform: methanol mixture and shaken with NaCl to remove non-lipid contaminants and treated for gravimetric estimation of lipids.

Mineral analysis

Samples for the determination of mineral content were subjected to both ashing as well as wet (nitric/sulphuric acid mixture) digestion methods. Ashing was done by incineration of seed samples (5gm) in muffle furnace at 600°C for 8 hours till seeds are converted to white ash. Ash content was weighed and treated with 5 ml conct. HCl in 100 ml volumetric flask and final volume was made up. However, wet digestion was done by treating the samples in triacid digestion mixture (conc. HCl, $HClO_4$ and H_2SO_4 in 3:2:1 ratio) for 20 min. Both ashed and wet digested samples were analyzed for all the mineral elements. Total Fe and P were estimated colorimetrically by α'α' dipyridyl method[26], respectively while other mineral elements (Na, K, Mg, Ca and Mn) were analyzed by using Dionex ICS 1100 Ion Chromatograph system equipped with guard column (CG12A), analytical column (CS12A), and cation self-regenerating suppressor (CSRS 300) using 20 mM Methane Sulphonic Acid as an eluent. The ion chromatograph system was calibrated against a range of working standards of each element. On the other hand, the digested samples were analyzed for Zn and Cu using flame atomic absorption spectrophotometer. Calibration of the instrument was done against a range of working standards of each element. Test solution was aspirated and the concentration of each element determined. The entire analysis was carried out in three replicates and the average values are reported.

Analysis of Vitamin A and C

For the determination of vitamin C, seed samples were extracted in metaphosphoric acid by centrifuging it at 5000rpm for 20 min and the supernatants was titrated against dye (2, 6-dichlorophenol indophenol) solution till the appearance of light pink colour. For standard 20 ml of ascorbic acid solution, volume of dye reduced was recorded. On the other hand, total carotene was extracted in petroleum ether-acetone (1:1). Vitamin A was separated by column chromatography and estimated colorimetrically[27]. Standard Curve was prepared by taking 0.2 to 2.0 ml of standard β-carotene solution in 50ml petroleum ether: acetone mixture and absorbance was taken at 450 nm.

Preparation of extract and Preliminary Phytochemical screening

The fine powdered seeds were extracted with hexane to remove lipids. It was then filtered and the filtrate was discarded. The residue was successively extracted with ethyl acetate and methanol using cold percolation method. The percentage yields were 2.1, 1.8. 1.6. 1.6 % in ethyl acetate and 10.8, 9.1, 8.8, 8.9

% in methanol for C. quinoa and C. album A, B and C, respectively.

For preliminary phytochemical screening, 1gm of the ethyl acetate and methanol extracts of C. quinoa and C. album seeds were dissolved in 100 ml of its own mother solvent to obtain a stock of concentration 1% (v/v). These extracts were subjected to the screening of alkaloids, amino acids, anthraquionones, flavonoids, gycosides, phytosterol, saponins, steroids, tannins and triterpenoids. The screening was done by following the methodology of[28,29].

Extract preparation and determination of total phenolic, tannin, flavonoid contents

Fine powdered seed samples (2 g) were homogenized in 70% aqueous acetone at room temperature and centrifuged for 15 min at 10,000 g and the supernatant was saved. The residue was re-extracted twice with 70% aqueous acetone (50 mL). The extracts were rapidly vacuum-filtered and refrigerated until assayed.

Total phenolics were determined by the Folin-Ciocalteu procedure[30]. Aliquots (0.1 mL) of aqueous acetone extracts were transferred into test tubes and their volumes made up to 0.5 mL with distilled water. After addition of Folin-Ciocalteu reagent (0.25 mL) and 20% aqueous sodium carbonate solution (1.25 mL), tubes were vortexed and after 40 min the absorbance of the resulting blue colored mixtures was recorded at 725 nm using UV – VIS spectrophotometer (Shimadzu, 4800) against a blank containing only extraction solvent (0.1 mL). The amount of total phenolics was calculated as a catechin equivalent from the calibration curve of catechin standard solutions (covering the concentration range between 0.1 and 1.0 mg mL^{-1}), and expressed as mg catechin/100 g dry plant material.

Total tannin content was determined by the Folin-Ciocalteu procedure as above, after removal of tannins by adsorption on an insoluble matrix (polyvinylpolypyrrolidone, PVPP). Insoluble, cross linked PVPP (Sigma, Germany; 100 mg) was weighed into test tubes and aqueous acetone extracts (1.0 mL) added. After 15 min at 4 °C, tubes were vortexed and centrifuged for 10 min at 4500 g. Aliquots of supernatant (0.2 mL) were transferred into test tubes and non-absorbed phenolics determined as described. Calculated values were subtracted from total phenolic contents and total tannin contents expressed as mg catechin/100 g dry plant material.

Total flavonoids were estimated using the method of[31]. To 1 mL of sample, 1 mL of 2% $AlCl_3$ ethanol solution was added. After 1 hour at room temperature the absorbance of yellow coloured solution was recorded at 420 nm against a blank containing only extraction solvent (0.1 mL). The amount of flavonoids was calculated as a quercetin equivalent from the calibration curve of quercetin standard solutions, and expressed as mg quercetin/ 100 g dry plant material[32].

Determination of saponin, phytic acid protease inhibitors

Saponin content was obtained by following the method of[33]. Seed samples were extracted several times in 20% ethanol. The extracts were reduced over water bath at about 90ºC and the concentrate was mixed with diethyl ether into separator funnel and shaken vigorously. The aqueous layer was recovered every time with the purification process. The purified extracts were mixed in n-butanol and washed twice with 5% aqueous sodium chloride. The remaining solution was heated in a water bath and after evaporation the samples were dried in the oven to a constant weight and the saponin content was calculated as percentage.

The presence of protease inhibitors was determined by a method described in[10]. Seed samples were treated with distilled water with continuous stirring for 16 h and the insoluble material was centrifuged at 10,000g for 20 min and discarded. A 0.8% agarose solution prepared in 0.1M Tris-HCl buffer (pH 8.6) was used to cast 2 nm thick gels which were punched into wells. After filling the wells with 5µl of test solution the slides were incubated in a chamber for 20 min at 37°C to allow inhibitor diffusion. The gels were immersed in 0.1M Tris-HCl buffer of pH 8.6 containing trypsin (100µg/litre). The gels were then washed 3 times with distilled water, and were incubated for 5 min at 37°C with excess of a substrate solution of 3 mg of N-acetyl-DL-phenylalanine 2-naphthyl ester dissolved in 1.25

ml of *N, N* dimethylformamide and mixed with 11 ml of 0.1M Tris-HC1 buffer of pH 8-6 containing 6 mg of tetrazotized o-dianisidine. Finally, the gels were transferred to 7.5% acetic acid to fix the colour. Phytate was quantified as hexaphosphate equivalents, using the method described in[10]. The seed samples were mixed with HCl (2.4%) by shaking vigorously for 3 h at 20°C. The suspension was filtered with vacuum through a What man No.1 paper and mixed with 1 ml of EDTA/NaOH solution (0.11M Na_2EDTA and 0.75M NaOH in water) and placed on the ion exchange (anion-exchange resin AG1-X4, 100-200 mesh chloride form) column (0.7 cm × 15 cm). The column was washed H_2O and 0.1M NaCl before it was eluted with 0.7M NaCl. The eluate was collected in a digestion vessel. A blank was prepared by mixing 2.4% HCl with Na_2EDTA-NaOH reagent and diluting it with water. A mixture of concentrated H_2SO_4 and HNO_3 was added to the eluate to release P by wet digestion in a Kjeldahl rack until a cloud of thick yellow vapour filled the neck of the flask. The content was then heated for 5 min at about 150°C, followed by heating for 5 min at about 80°C. The samples were cooled to room temperature and then H_2O was added to dissolve the salts. They were again heated for 10 min under low heat (about 80°C). After cooling, 2.5% ammonium molybdate solution in 1N H_2SO_4 was added and mixed well, and then 1 ml of sulphonic acid reagent (0.16 g of 1-amino-2- naphthol-4-sulphonic acid, 1.92 g of Na_2SO_3 and 9-6 g of $NaHSO_3$ in 100 ml of H_2O) was added in the solution. After mixing well, the solution was allowed to stand 15 min and the absorbance was measured at 640 nm. The amount of phytate (phytate = 28.2% P) in the sample was calculated as hexaphosphate equivalents. Sodium phytate solutions were used as standards.

Determination of anti-oxidant activity

In order to measure antioxidant activity DPPH free radical scavenging assay was used. This assay measures the free radical scavenging capacity. DPPH is a molecule containing a stable free radical. In the presence of an antioxidant, which can donate an electron to DPPH, the purple color which is typical for free radical decays and the absorbance was measured at 517 nm using a double beam UV-VIS spectrophotometer. The ethanolic extracts of the plants were re-dissolved in ethanol and various concentration (50, 100, 150 and 200 µg/ml) of extracts were used. The assay mixture contained in total volume of 1 ml, 500µl of extract, 125µl prepared DPPH and 375 µl solvent (ethanol). After 30 min of incubation at 25°C, the decrease in absorbance was measured at 517 nm on spectrophotometer against a blank containing absolute ethanol (0.5 mL) instead of a sample aliquot. All measurements were done in triplicate.

The radical scavenging activity (RSA) was calculated as a percentage of DPPH using a discoloration using then equation % RSA = $[(A_0 – A_s)/A_0]$ x 100, where A_0 and A_s are the absorbance of control and test sample, respectively.

Statistical analysis

The results were reported as means of at least three measurements, each performed in triplicate. One way analysis of variance (ANOVA) was used to compare the means and the least significant difference (LSD) test showed the values statistically different. Differences were considered significant at $P < 0.05$. All statistical analysis was performed with SPSS 16.0 software.

RESULTS

Proximate composition

Although *C. quinoa* is a lesser-known plant but there has been increasing interest due to its perceived superior nutritional quality compared to other grains. The proximate composition of quinoa ranges from 10 to 18% for protein, from 4.5 to 8.75% for crude fat, from 54.1 to 64.2% for carbohydrates, from 2.4 to 3.65% for ash and from 2.1 to 4.9% for crude fiber[34]. However, Ando[6] reported that quinoa seeds contain carbohydrates (77.6%), protein (12.9%), balanced amino acid spectrum of high lysine and methionine contents, lipids (6.5%) and is rich in dietary fiber.

In the present study, the proximate composition of the seed samples (*C. album* and *C. quinoa*) examined is presented in Table No.1. All samples contained between 11.3 - 13.2% moisture and 2.1 - 3.2% ash. The protein content ranged from 3 to 5.5 g/100g with

highest content (5.5 g/100g) in *C. quinoa* seeds followed by *C. album* A (3.4 g/100g). The values of protein in *C. album* seeds in present study are little lower than the values of protein in leaves (3.69%) as reported by[20]. However, earlier reported literature shows high content of protein in quinoa seeds (12-19 %)[35]. The lower values in the present study are probably because the seeds used in the present study were commercially obtained and are debittered seeds in which the protein seed coat was removed. Reducing sugar in all the four varieties ranges between 2.2 to 3.1%. Among the *C. album* samples, seeds of sample A contain highest percentage of reducing sugar (2.6 g/100g) which is equal to the reducing sugar content of *C. album* C (2.5 g/100g) indicating that wild seed samples have high content of sugars. The seeds of *C. quinoa* were found to be a good source of total sugar content which was found to be 7.7 g/100g. Among all the *C. album* samples, cultivated seed samples (*C. album* B) contained lowest amount (5.1%) of total sugar while sample A contained highest amount (5.5%) of total sugar. Lipid content in all the seed samples ranged from 4.5 to 5.8 g/100g and found to be higher in *C. album* A (5.8 g/100g) as compared to the seeds of *C. quinoa*. Przybylski[36] isolated and characterized lipids from quinoa seed and found the largest amount (20%) of neutral lipids was contributed by the diglycerides. The overall fatty acid compositions of whole quinoa seeds contain linoleic, oleic and palmitic acids as the major acids which were found to be similar to other cereal grains.

Vitamin and mineral levels

Mean values for mineral content of nutritional importance are presented in Table No.2. The order of minerals found in the seed samples were in the order of K > P > Ca > Mg > Na > Fe > Zn > Mn > Cu. All the seed samples analyzed in the study were excellent source of K, P and Ca content. The K content varied greatly and ranged from 481.4 mg/100g (*C. album* B, cultivated) to 865.2 mg/100g in *C. quinoa*. On the other hand, the levels of P and Ca were found to be higher in the seeds of *C. quinoa* (556.8 mg/100g of P and 197.8 mg/100g of Ca) followed by the seeds of *C. album* A > *C. album* B > *C. album* C. On comparison, the levels of Mg were found to be equal in all seed samples of *C. album* while the levels were found to be much higher in *C. quinoa*. The highest quantity of sodium (34.8 mg/100 g) was found in the seeds of *C. quinoa* while it was found to be nearly equal in all the seed samples of *C. album*. The richest source of zinc was found in *C. quinoa* (2.8 mg/100 g) while it was found to be lowest in C. album (1.5 mg/100 g). Mn was found in the narrow range of 0.8 to 1.8 mg/100g in all seed samples of *C. album* and *C. quinoa*. Cu content in all the seeds was observed in traces ranging from 0.01 to 0.08 mg/100g in all seed samples of *C. album* and *C. quinoa*. The seed samples evaluated contained substantial quantities of iron and the seeds of *C. quinoa* contained a significantly high level of iron (9.8 mg/100 g).

The results obtained indicated appreciable amount of vitamin A content in *C. album* seed samples as compared to *C. quinoa* seeds. In the seed samples of *C. album*, the levels of vitamin A ranged from 4.89 mg/100g to 6.61 mg/100g while it was found to be very low in the seeds of *C. quinoa.* No significant difference was found in vitamin C content and it ranged from 10.2 mg/100g to 12.8 mg/100g in all the seed samples of *C. album* and *C. quinoa*. Raju[23] observed high levels of β carotene and lutein in the leaves of C. album (114.61 mg/100 g dry wt. and 187.59 mg/100 g dry wt.) as compared to other green leafy vegetables.

It is interesting to note that although *C. album* is a wild species and is not a cereal but it contained significant levels of all minerals and vitamins, thus it can be used as an alternative source to meet the nutritional demands of especially poor and rural peoples.

Phytochemical screening

Phytochemicals with biological activity are known to have valuable therapeutic index[29]. Phytochemicals such as saponins, terpenoids, flavonoids, alkaloids, tannins and steroids have anti– inflammatory effects[37,38]. Glycosides, flavonoids, alkaloids and tannins have hypoglycemic activities[39,40]. Studies also show that saponins have antidiabetic while steroids and triterpenoids show analgesic properties[41,42].

In the present study, the ethyl acetate and methanol extract of the seeds of *C. album and C. quinoa* showed the presence of different phytochemicals as shown in Table No.3. Almost all the analyzed phytochemicals were present in the all the seed samples except anthraquinones. Thus, the seeds of *C. album and C. quinoa* may also have various medicinal values such as anti - inflammatory, antidiabetic and analgesic activities.

LEVELS OF ANTI-NUTRIENTS

The levels of various antinutrients like saponins, tannins, phenolics etc. present in the seeds of *C. album* and *C. quinoa* were determined and reported in (mg/100g) on dry weight basis in Table 4.

Saponins

Saponins are the major antinutritional factors present in the seed coat of *C. quinoa*[7]. Saponins are glycosidic triterpenoids or sterols that are widely distributed throughout the plant. Their concentrations vary among plants; they occur usually within a range of 0.1 to 5.0%[43]. Saponins are toxic and their toxicity depends upon the type of saponin and the method of absorption. Due to their differential toxicity with respect to various organisms, saponins are also used as natural insecticides without adverse effects on higher animals and humans. Saponins have immense industrial importance and are used in the preparation of soaps, detergents, shampoos, beer, fire extinguishers and photography, cosmetic and pharmaceutical industries[44]. Another interest in saponins is with their antibiotic, fungistatic, and pharmacological properties[45]. The pharmacological interest in saponins concerns their ability to induce changes in intestinal permeability which may aid the absorption of particular drugs and their hypocholesterolemic effects[46]. However, because saponins may impart a bitter taste, the separation of saponins from quinoa seed is easily accomplished by rinsing the seed in cold alkaline water or by mechanical abrasion[47]. The protein quality of a saponin-free quinoa has shown better growth response than white rice, maize or wheat[34].

Saponins isolated from seeds of C. quinoa have shown that they exert antimicrobial activity[48,49], toxicity to brine shrimps and against viral diseases, cholesterol-lowering effects, and enhances drug absorption through mucosal membranes. They act as immunological and absorption adjuvants to enhance antigen- specific antibody and mucosal responses[50]. Zhu[51] isolated 12 triterpene saponins from debittered seeds of C. quinoa and reported that the saponin concentrations ranged from 0.4 to 5.6% on a dry weight basis with a mean value of 2.2%. On the other hand, in the present study it ranges from 0.21 to 0.32 % on a dry weight basis of *C. sp*. The highest concentration was observed in the seeds of C. quinoa (0.32%) followed by C. album A (0.27%), B (0.25%) and C (0.21%) on a dry weight basis.

Phytic acid

Phytic acid [myoinositol 1, 2, 3, 4, 5, 6-hexakis (dihydrogen phosphate)] is an important antinutritional compound present in many cereals at concentrations of l-3% by weight. It is present in the outer layers of wheat and rye but in case of quinoa seeds phytic acid is evenly distributed in the outer layers as well as in the quinoa endosperm[10]. In the gastrointestinal tract, it can form insoluble complexes with multivalent cations such as iron, zinc, calcium and magnesium rendering them unavailable in food[52]. The reduction of phytic acid in foods by processes like fermentation, sprouting and scalding has been reported[52].

The phytic acid concentration ranged from 1.05-1.31g/100 g in five different varieties of quinoa[43] with the mean concentration of 1.18g/100 g which was found to be similar as compared with other cereals like barley 1.07g/100 g, maize 0.94g/100 g, rice 0.89g/100g and wheat 0.99g/100g[53]. Although quinoa has more phytic acid than the cereals, no adverse effects were seen on the incorporation of calcium into bone or on iron absorption[43]. The concentrations of phytic acid ranges from 0.74 - 0.81 g/100 g in C album A, B and C, respectively (Table No.4). However, the content of phytic acid obtained in quinoa was found to be 0.9 g/100 g which was found to be comparable with the values (1.04 ± 0.08 g/100 g) reported by[10]. The process of removing the bitter taste from quinoa and album seeds was carried out by first scrubbing and then washing, leading to a significant decrease (about 35%) in the recovery of

phytic acid. The phytic acid concentration in processed quinoa seeds was comparable with those values for whole grain rye flour (0.77 g/100g), whole grain wheat flour (0.87 g/100g), lentils (0.84 g/100g), kidney bean (0.8 - 1.2 g/100g) and faba bean (0.8 g/100g)[54].

Tannins

Tannins measured as catechin mg/100g of dry weight plant material were found in appreciable good amounts in both the species of Chenopodium. It ranged from 115 - 220 mg/100g and was found highest in *C. quinoa*. In case of *C. album*, tannin concentration was found to be highest in *C. album C* (121 mg/100g) followed by *C. album A* (119 mg/100g) on dry weight basis. However, Koziol[43] does not found any tannin content in raw whole quinoa or raw polished and washed quinoa. On the other hand, Chauhan[55] reported 0.53 g of tannins in whole quinoa seeds, 0.28 g in manually dehulled (flour) and 0.23 g in water dehulled (flour) per 100 g on a dry matter basis. This variation in tannin levels may be due to the variety and growing conditions of the plant analyzed. On comparison, we observed that tannins were present in sufficient amounts in many seeds like soya bean (g*lycine max*) 0.05%, *amaranthus paniculatas* 0.04 - 0.13%, kidney bean (p*haseolus vul*) 1.02%, rice beans (1.3%), green gram (1.1%), black gram 1.1% and barley 0-12%[55,22,10].

Protease inhibitors

The concentration of protease inhibitor in the seeds of *C. album* and *C. quinoa* was below the detection limit (BDL) of the qualitative method used for the determination of trypsin inhibitor. The results were found to be similar as reported by[10]. He concluded that the protease inhibitors in quinoa seeds are below 50 ppm (0.97 TUI/100 g where TUI is Trypsin units inhibited. On comparison, we observed that protease inhibitors were present in sufficient amounts in many seeds like soya bean (g*lycine max*) 24.5 - 41.5 TUI/mg, *amaranthus paniculatas* 0.5 TUI/mg, kidney bean (p*haseolus vul*) 12.9 - 42.8 TUI/mg and lentils (l*ens esculenta*) 17.8 TUI/mg[10,22]. However, as indicated in table 4, the trypsin inhibitor units are absent in *C. album* and *C. quinoa*, thus retain the bioavailability of protein in both the species of Chenopodium.

Flavonoids

Flavonoids, one of the typical polyphenols present in vegetables, fruits and tea, can prevent degenerative diseases such as coronary heart disease[56], atherosclerosis, cancers, diabetes, and Alzheimer's disease[57], through antioxidative action and/or the modulation of several protein functions[15]. Higher concentrations of flavonoid derivatives also enhance the neutraceutical value in terms of health-promoting effects. Quercetin is the strongest antioxidant among flavonoids; which have diverse biological effects. Several examples of plants rich in quercetin are onions (21.4 mg/100 g), green tea leaves (dry, 662 mg/100 g), capers (234 mg/100 g), lovage leaves (170 mg/100 g), buckwheat (23.1 mg/100 g), etc. Hirose[15] reported that quinoa seeds possess large amounts of quercetin and kaempferol glycosides and the quinoa seeds cultivated in Japan are the most effective functional foodstuff in terms of being a source of antioxidative and bioactive flavonoids. In the present study, the flavonoid concentration ranged from 83 mg/100 g in *C. album B* to 108 mg/100 g in *C. quinoa* which indicated that besides supplying the basic nutritional requirements, the seeds of *C. sp.* also have health-promoting and/or disease-preventing properties.

Anti - oxidant activity and total phenolics

The DPPH radical has been widely used to test the potential of compounds as free radical scavengers of hydrogen donor and to investigate the antioxidant activity of plant extracts. The ethanolic extract of seed samples of Chenopodium species showed moderate free radical scavenging in DPPH assay. The extract of *C. quinoa* exhibited antioxidant effect at low concentration. When the extract of the seeds was tested for DPPH radical scavenging activity, it was found that 50 µg/ml and 100 µg/ml of the extract lowered the DPPH radical levels above 46 and 58 % respectively. On the other hand, the extract of plant *C. album* A, B and C showed low antioxidant property but at higher concentration which was found to be 9.6 and 22 % at the concentration of 150 µg/ml and 200 µg/ ml respectively. The results indicated that the ethanolic seed extracts of *C.*

quinoa showed good antioxidant activity in comparison to *C. album* A, B and C on DPPH free radical. Significant differences ($p < 0.05$) in were observed in the seed samples of *Chenopodium* species. Nsimba[58] evaluated the antioxidant potency of various extracts and fractions from *C. quinoa* using the DPPH scavenging activity and observed that seeds can act as powerful dietary antioxidants. Some authors have also reported low anti –oxidant activity in the leaves of *C. album*[22,59].

Total phenolics content in the seeds of *C. album* and *C. quinoa* is shown in Table No.4. It varied from 189 mg catechol/100 g dry weight in *C. album C* to 224 mg catechin/100 g dry weight in *C. quinoa*. The difference in the phenolics concentration between the three ecotypes of *C. album* might be explained by the difference in the environmental conditions or genetic background. The antioxidant activity was less correlated to the phenolics content ($r^2 = 0.27$) suggesting that non-phenolic compounds might play major free radicals scavenging activity in studied plant materials. Kaur and Kapoor[22] reported high phenolic content (253.5 mg/100g) in the leaves of *C. album*. Jafari and Kholdebarin[60] investigated the allelopathic effects of *C. album* and concluded that complete nitrite oxidation to nitrate (nitrification) was delayed due to high phenolic content in *C. album*. The plant also releases phenolics into the soil and these are probably involved in the growth inhibitory effect of *C. album*[61].

Table No.1: Proximate composition in the seeds of *C. album and C. quinoa* in (g/100g)

S.No	Seed Samples	Moisture	Ash	Protein	Carbohydrates		Lipids
					Reducing Sugar	Total Sugar	
1	*C. album* A	11.3	2.4	3.4 ± 0.5	2.6 ± 0.3	5.5 ± 0.3	5.8
2	*C. album* B	12.1	2.1	3.2 ± 0.4	2.2 ± 0.2	5.1 ± 0.1	4.5
3	*C. album* C	13.2	2.2	3.0 ± 0.6	2.5 ± 0.1	5.3 ± 0.2	5.1
4	*C. quinoa*	12.3	3.2	5.5 ± 0.5	3.1 ± 0.1	7.7 ± 0.4	5.6

Table No.2: Minerals and vitamin content in the seeds of *C. album and C. quinoa* in (mg/100g)

S.No	Seed Samples	Na	K	Mg	Ca	Mn	Fe	P	Zn	Cu	Vitamin A	Vitamin C
1	*C. album* A	22.9	512.8	133.8	158.6	1.1	5.7	463.5	1.7	0.02	6.61	11.3
2	*C. album* B	22.1	481.4	131.7	152.6	0.8	5.1	462.9	1.5	0.01	5.21	10.2
3	*C. album* C	21.9	488.5	135.3	147.4	1.0	4.9	450.8	1.9	0.02	4.89	10.7
4	*C. quinoa*	34.8	865.2	189.6	197.8	1.8	9.8	556.8	2.8	0.08	3.07	12.8

Table No.3: The analysis of phytochemicals in the ethyl acetate and methanol extract of the seeds of *C. album and C. quinoa* showing presence (+) and absence (-)

S.No	Phytochemicals	*C. album* A	*C. album* B	*C. album* C	*C. quinoa*
1	Alkaloids	+	+	+	+
2	Amino acids	+	+	+	+
3	Anthraquinones	-	-	-	-
4	Flavonoids	+	+	+	+
5	Glycosides	+	+	+	+
6	Phytosterol	+	+	+	+
7	Saponins	+	+	+	+
8	Steroids	+	+	+	+
9	Tannins	+	+	+	+
10	Triterpenoids	+	+	+	+

Table No.4: Anti nutrient content in the seeds of *C. album and C. quinoa* on dry weight basis

S.No	Seed samples	Saponin (%)	Phytic acid (mg/g)	Tannins (mg/100g)	Phenolics (mg/100g)	Flavonoid (mg/100g)	Trypin units inhibited (TUI/mg)
1	*C. quinoa*	0.32	9	220	224	108	BDL
2	*C. album A*	0.27	7.4	119	194	84	BDL
3	*C. album B*	0.25	7.9	115	189	83	BDL
4	*C. album C*	0.21	8.1	121	191	86	BDL

CONCLUSION

The results of the study imply that the seeds of *Chenopodium album* are at par with the *C. quinoa* seeds and well-endowed with essential nutrients required for human consumption. The seeds of both species of *Chenopodium* were moderately nutritive not only in terms of lipids, carbohydrates and proteins but also a remarkable source of minerals and vitamins. In the light of results obtained from the total phenolics, flavanoids and anti - oxidant activity, the seeds of *C. album* and *C. quinoa* also have health-promoting and/or disease-preventing properties, besides supplying the basic nutritional requirements. Thus, the *C. album* growing as a weed in the fields of wheat and gram can be used as a future crop and is recommended for future commercial cultivation.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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