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# Evaluation of some Quinoa genotypes on the production of active constituents and cytotoxic activity

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## Abstract

In the last two decades, quinoa has gained great popularity in many countries of the world due to its numerous agricultural and medicinal benefits. This paper highlights two objectives: first, to evaluate the differences between three genotypes of quinoa (*Chenopodium quinoa* Willd.) in terms of crop and chemical characteristics and to study the association between them, and second, to what extent extracts of these genetic constructs may be effective in the treatment of liver cancer. The results indicate that there are significant differences for the tested traits except for globulin (%), 1000 grain weight. The results also indicate that there is a strong positive correlation between the yield, plant height and total protein (%), as well as a strong correlation between protein and both albumin a and albumin b, which enhances the focus on these traits in selecting the distinctive genotype in the grain yield and its protein percentage. HPLC analysis of the ethanol extract of the seeds of the three genotypes revealed that reveals that the major flavonoid and phenolic in chipaya is quercetin (102.20 µg/g) and vanillin (98.32 µg/g) respectively. However, in L14 is naringenin (10.04 µg/g) is the major flavonoid and syringic acid the highest phenolic acid (68.88 µg/g). Concerning the genotype Q5 is hesperetin (22.19 µg/g) is the major flavonoid and chlorogenic acid (78.95 µg/g) the highest phenolic acid. In vitro cytotoxic activity of the three genotypes against hepatocellular carcinoma cell line (HPGE2) indicated that all extracts inhibits the viability and the $IC_{50}$ of the three Genotypes chipaya, L14 and Q5 were 214.37 ± 3.91, 114.99±0.46 and 113.72 ± 0.87 µg/ml respectively.

## 1. Introduction

Many plants, including quinoa, have occupied a distinguished position due to their diverse adaptation mechanisms to different environmental conditions in addition to their unique nutritional value. Therefore, attention is directed towards using this crop as a good model for developing the agricultural sector or achieving economic profits in the Middle East region (1-3).

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*Chenopodium. quinoa* Willd. a pseudo cereal that has been cultivated in the Andean region of South America for thousands of years and belongs to the Chenopodiaceae family (4-5).

Quinoa is grown in more than 100 countries, with the largest production in Peru and Bolivia (90%). Global quinoa production has reached about 175,000 tons, and the United States is the largest consumer and importer of quinoa in the world. Quinoa grains do not contain gluten while containing a high percentage of protein, essential amino acids, minerals and vitamins (6) compared to major cereals as wheat, corn, rice, and barley (7-8). The average protein content of quinoa seeds ranges from 12 to 23% depending on the genetic makeup (9). Quinoa grains are also rich in bioactive compounds (flavonoids, phenolic acids, saponins, bioactive peptides and phytosterols (10-11).

Previous studies indicate that the main proteins in quinoa seeds are 37% globulins, 35% albumins, and a limited proportion of prolamins (0.5–7.0%) (12-13). Recently, quinoa seeds have been used as a source of gluten-free and healthy flour for celiac patients (14).

Previous studies have shown that the compositional evaluation of quinoa shows that its proteins are mainly stored in the embryonic tissues which also contain lipids, fibers, ash and saponins while the outer seed coat is rich in starch (15-16). Proteins also contribute mainly to the structural and functional properties of quinoa, such as solubility, gel network formation, foaming and emulsifying properties (17-18).

It was observed by Pasko et al. (19) that bioactive compound of quinoa could prevent the oxidative stress and also helps in decreasing the risk of various chronic diseases such as inflammation, immune disease, and cancer (20). The antioxidant effect of quinoa seeds is evident in their cardioprotective role by improving and normalizing the values of cardiac enzyme markers, LDH and CK that significantly increased their enzymatic activity after treatment with doxorubicin (21). Quinoa is important as an inexpensive and unique natural source of anti-diabetes, thus it is a promising complementary therapeutic agent against diabetes (22).

The promising importance of quinoa economically, phytochemically and biologically, made researchers and producers to search for new genotypes having better characters. This led to the idea of this article which is evaluating the phytochemical constituents and in-vitro anticancer activity of three new genotypes.

## 2. Materials and Methods

### *2.1. Plant material:*

Three genotypes of *Chenopodium. quinoa* Willd. were obtained as follows: Chipaya genotype as commercial cultivar from Desert Research Center in Egypt while the second genotype (Line 14) from N.B.R.I., Lucknow in India and Q5 cv. from International Center for Biosaline Agriculture in United Arab Emirates. The genotypes were grown under field conditions during the successful winter growing season 2022/ 2023 in Ismailia region, Egypt.

#### *2.1.1. Measurements of vegetative, agronomic and chemical characters.*

- Plant height (cm) of plant.
- Amount of chlorophyll was measured using chlorophyll Meter SPAD-502 where, the mean chlorophyll content (SPAD) of the tested quinoa genotypes were taken after 60 days of cultivation where the chlorophyll content was read in three leaves (6th, 7th and 8th from the plant apex) from five plants from each replicate.
- Weight of 1000 grain (g).
- Grain yield / fed. (Kg).
- Chemical properties (Estimation of total protein, albumins a,b and globulins): total protein was determined as described in AOAC International (23). To convert nitrogen content to crude protein content, a factor of 6.25 (N factor) was used. Proteins were removed sequentially according to their solubility; albumins (a,b), globulins (Glo). For extraction, 1 g of ground seeds was weighed and the corresponding solvent was added (ratio 1:10 w/v): water, phosphate buffer solution pH 7.5, 70% ethanol, and 0.1 M sodium hydroxide, respectively. After each extraction, samples were centrifuged at 11,000 g for 15 min at 4°C, and the upper liquid was collected and stored at 2°C until further use. Protein content was determined by the Bradford method (24). All determinations were made in three replicates.

Residual = 100 – (albumins a+ albumins b + globulins)

#### *2.1.2. Statistical analysis*

Statistical analysis was performed using standard procedures for randomized complete block design, by analysis of variance (ANOVA). Separation of means was performed by applying Duncan's mean difference comparisons at a significance level of $p \leq 0.05$. SAS University Edition software was used (SAS 2014) (25). The data were expressed as the mean of three biological replicates.

The correlation coefficient analysis was performed between all tested traits according to Singh and Chaudhary (26).

### *2.2. Quinoa seed extraction process*

Quinoa seeds (Chipaya, L14 and Q5 genotypes) were washed and left to dry for 24 h in a drying cabinet set at 60 ◦C. The dried quinoa seeds were ground and ten gram of the ground seeds were subjected to extraction 70% ethanol. Then filtered with coarse filter paper and then ethanol was evaporated at 50 ◦C in a rotary evaporator to obtain quinoa seed extract. The extracts were kept for further chemical and biological investigation.

*2.3. Quantitative estimation of flavonoids and phenolic using HPLC*

*2.3.1. HPLC conditions*

HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using Zorbax Eclipse Plus C8 column (4.6 mm x 250 mm i.d., 5 µm). The mobile phase consisted of water (A) and 0.05% triflhuoroacetic acid in acetonitrile (B) at a flow rate 0.9 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–1 min (82% A); 1-11 min (75% A); 11-18 min (60% A); 18-22 min (82% A); 22-24 min (82% A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 5 µl for each of the sample solutions. The column temperature was maintained at 40 °C.

*2.4. In vitro cytotoxic activity:*

*2.4.1. Measurement of the potential cytotoxic activity:*

was performed on liver carcinoma cell lines (HEPG2), using the ethanolic extract of *Chenopodium quinoa* Willd. seeds extract (Chipaya, L14, Q5), while doxorubicin was used as a positive control. The procedures were according to (MTT protocol) Viability assay (27-28).

*2.4.2. Morphological assay:*

Large-scale, morphological changes that occur at the cell surface, or in the cytoskeleton, can be followed and related to cell viability. Damage can be identified by large decreases in volume secondary to losses in protein and intracellular ions of due to altered permeability to sodium or potassium. Necrotic cells: nuclear swelling, chromatin flocculation, loss of nuclear basophilia. Apoptotic cells: cell shrinkage, nuclear condensation, nuclear fragmentation (29).

*2.4.3. Statistical analysis:*

Each value is the mean of three replicates. Obtained values were presented as mean ± SD. Significant differences between the values were calculated using SPSS software (V.22) using One-way ANOVA and Tukey's test. The difference is considered significant at ($P<0.05$). To compare the $IC_{50}$ value of Chipaya, L14, Q5 and doxorubicin t-test was performed the difference was considered significant at ($P<0.05$).

## 3. Results

The results in Table (1) showed the performance means of some quinoa plant traits and significance for the three tested genotypes. The data showed significant differences in plant height, chlorophyll content in leaves, and grain yield per feddan, while the results showed no significant difference between the three genotypes for 1000 grain weight trait. The results also show that both Chipaya and L14 cultivars outperformed Q5 cultivar compared with general mean (870kg/fed.).

*3.1. Total protein, globulin and albumin content*

The data in Table 2 showed significant variation in total protein content among the tested genotypes during the 2022/2023 agricultural season, as the highest protein content was in the seeds of Chipaya genotype, while Q5 genotype recorded the lowest total protein content.

Albumin a, b content was the highest in the Chipaya genotype followed by the L14 genotype, while the Q5 genotype recorded the lowest percentages of albumin a and b (11.35 and 1.22, respectively). As can be seen from the table, albumin (a) was higher than albumin b in the three tested genotypes.

While there was no significant difference in globulin content ($p \leq 0.05$) between the three tested models during this season (Table 2). In general, the seed content of globulins was the main part in mature quinoa seeds, as the production of globulins was higher than albumins (Table 2).

the other hand, the residuals percentage of total protein (excluding albumin and globulin) showed a significant difference between the Chipaya genotype and the other two genotypes (L 14 and Q5) while there was no significant difference between L14 and Q5 genotypes.

*3.2. Correlation coefficient*

The results of the Table 3 indicate that most of the traits have a significant correlation, except for the correlation between chlorophyll content in leaves and plant height, globulin and grain yield per feddan, which shows a non-significant correlation.

The data also shows a negative significant correlation between the weight of 1000-grain trait and all other traits. On the other hand, the data showed that the highest positive significant correlation was recorded between grain yield per feddan and plant height (1.000), between albumin (a), albumin (b) (0.999), and also between albumin (a) and protein %.

*3.3. HPLC analysis:*

The HPLC analysis of three *Chenopodium quinoa* Willd. genotypes (Chipaya, L14 and Q5) as shown in table (4) reveals that the major flavonoid and phenolic in chipaya is quercetin (102.20 µg/g) and vanillin (98.32 µg/g) respectively. However, in L14 is naringenin (10.04 µg/g) is the major flavonoid and syringic acid the highest phenolic acid (68.88 µg/g). Concerning the genotype Q5 is hesperetin (22.19 µg/g) is the major flavonoid and chlorogenic acid (78.95 µg/g) the highest phenolic acid. Rutin is absent in the three genotypes. While, chlorogenic acid is absent in chipaya. Ellagic acid is absent in both chipaya and L14. Coffeic acid is absent in Q5. On the other hand kampferol and catechin are absent in both L14 and Q5.

*3.4. Cytotoxic activity:*

Figure (1) shows that the viability of the hepatic carcinoma cell lines (HEPG2) decreases by increasing the

concentration of the extract of the three *Chenopodium quinoa* Willd. genotypes (chipaya, L14 and Q5). Figure (2) illustrates the cytotoxic activity of doxorubicin on liver carcinoma cell line (HEPG2). Table (5 and 6) shows the statistical analysis (One-way ANOVA and Tukey HSD / Tukey Kramer) of the surviving fraction of hepatic carcinoma cell line cells where the significant difference is calculated at $P< 0.05$).

Figure (3) (The $IC_{50}$ of chipaya, L14, Q5 and doxorubicin is calculated (214.37 ± 3.91, 114.99±0.46, 113.72 ± 0.87 and 15.22±0.07 µg/ml respectively). T-test analysis indicated that there is a significant difference between chipaya and both L14 and Q5 at $p <0.05$. From the previous data it could be concluded that chipaya is less active than L14 and Q5, where both of them are equivalent to each other as anticancer against hepatic carcinoma cell line. There is a significant difference between doxorubicin and the three genotypes.

Figures (4, 5 and 6) show the morphological changes in the hepatic carcinoma cell lines with different concentration (1000, 500, 250, 125, 62.5 and 31.25 µg/ml) of the seed extracts (chipaya, L14 and Q5). Figure (7) show the morphological changes in the hepatic carcinoma cell lines with different concentration (100, 50, 25, 12.5, 6.25 and 3.125 µg/ml) of doxorubicin.

## 4. Discussion

*Mean performance of traits and their correlation coefficient*

Considering the data in Table 1, 2 and Table 3, it becomes worth mentioning to discuss several points as follows:

Although there are significant differences between the chlorophyll content in the three quinoa genotypes, this trait is not positively correlated with all other traits, including yield, but this chlorophyll content is only positively correlated with the weight of 1000 grain (the differences between the three genotypes are not significant ), and therefore it can be said that this trait cannot be relied upon in selection for grains yield directly or indirectly through the weight of 1000 grains (Table 1, 3).

On the other hand, it is clear from Table 2, 3 that grain yield as a major trait can be selected among the tested genotypes through both plant heights strongly as well as the total protein ratio due to the relatively high correlation rate between them.

Despite the strong positive correlation between grains yield and the globulin trait, it cannot be relied upon in selection due to non-significant differences between the tested genotypes for this trait.

Protein content (%) can also be considered an important trait as the data showed a strong positive correlation between total protein content and both amino acids albumin a and albumin b (0.990 and 0.995, respectively) and can be used as an effective means of selecting genotypes with high protein content, this view is supported by the strong correlation between albumin a and albumin b (0.999).

Conversely, the positive correlation between protein and globulin (0.620) cannot be used directly as there are no statistically significant differences between the tested genotypes for the globulin trait.

Spehar and Santos (30) found a significant positive correlation between length of inflorescence in quina and grain yield, suggesting that selection for these traits may lead to the selection of more productive genotypes (31). For example, quinoa plants with more branching characteristics tended to develop larger inflorescences. Inflorescence length was also positively correlated with height of quinoa plant, suggesting that genotypes with greater plant height evolved taller inflorescences (32-33).

In general, this research agrees with what Badran et al. (34), confirmed that the data resulting from the evaluation of phenotypic traits and indicators of quinoa genotypes tolerance through the results of biochemical and molecular analysis provide new insights for breeding programs to improve quinoa to produce new genotypes that have the ability to confront environmental conditions and help reduce the food gap in the future.

*Cytotoxic activity*

Due to increasing frequency rates of liver cancer and concerns about the toxicity of current chemotherapeutic medicines, the quest for further substitutes to treat this carcinogenicity has enhanced. Liver diseases are caused by different etiological agents, mainly alcohol consumption, viruses, drug intoxication or malnutrition. Frequently, liver diseases are initiated by oxidative stress and inflammation that lead to the excessive production of extracellular matrix, followed by a progression to fibrosis, cirrhosis and hepatocellular carcinoma (35).

Chlorogenic acid inhibites the proliferation, colony formation, invasion, and metastasis of HepG2 cells both in vitro and in vivo. Chlorogenic acid can suppress liver cancer cell proliferation, invasion, and metastasis through several pathways. Chlorogenic acid could serve as a candidate chemopreventive agent for hepatocellular carcinoma (36-37).

Hesperetin was proved to be a promising naturally active agent for prevention of hepatocellular carcinoma. It Also, can be considered as a potent chemosensitizer for chemotherapeutic agents such as 5-fluorouracil. Hesperitin applied its antitumor effect mainly by induction of Fas/FasL extrinsic apoptotic pathway as well as, implemetation of the antioxidant defense mechanisms (38).

It has been reported that some natural products display hepatoprotective properties. Naringenin is a flavonoid that exhibits hepatoprotective effect. It has antioxidant, antifibrogenic, anti-inflammatory and anticancer properties that is capable of preventing liver damage caused by different agents. The main protective effects of naringenin in liver diseases are the inhibition of oxidative stress, transforming growth factor (TGF-β) pathway and decreased collagen synthesis. Naringenin has shown favourable influence on nonalcoholic fatty liver disease (NAFLD) through the

direction of lipid metabolism, regulating the synthesis and oxidation of lipids and cholesterol. As well as, it inhibits growth factors such as TGF-β and vascular endothelial growth factor (VEGF), thus motivating apoptosis. Naringenin is safe and can be considered in the future as an important candidate in the treatment of different liver diseases (35-39).

Syringic acid has a protective effect against hepatocellular carcinoma by reducing the liver marker levels (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alpha-fetoprotein (AFP), and gamma-glutamyl transferase (GGT)) and increasing the expression of apoptotic proteins (P53, Bax, Apoptosis regulator Bcl2, Caspase 3, Caspase 9, Cytochrome C, Tnf α, Nfκb and Traf1). A docking study proved that syringic acid has good anticancer activity (40).

Quercetin is a flavonoid relatively less harmful to normal cells in comparison to chemotherapy and is an excellent free-radical scavenger. It is present in many fruits, vegetables, and herbs. Quercetin can suppress liver carcinoma in vivo and in vitro. It inhibits the proliferation of liver cancer cells via induction of apoptosis and cell cycle arrest. Quercetin targets apoptosis, by upregulating Bax, caspase-3, and p21 while downregulating Akt, PLK-1, cyclin-B1, cyclin-A, CDC-2, CDK-2, and Bcl-2. Additionally, it has been reported to increase STAT3 protein degradation in liver cancer cells while decreasing STAT3 activation. Quercetin could be considered a novel potential anticancer drug candidate (41-43).

Vanillin (4-hydroxy- 3-methoxybenzaldehyde) plays important role in the process of inhibiting tumor growth. Four vanilloid receptors (one transmembrane channel *TRPV1* and three cytoplasmic peptides – MARK4, CAMK4 and CK2) play significant roles in the response of cancer cells to the natural compound. These vanilloid receptors inhibit the proliferation of cancer cells as response to vanillin and its derivatives (44).

## Conclusion

Studying the significance of the different parameters of genotypes and determining the correlation coefficient between them can contribute to identifying the most appropriate of these genotypes for multiple aspects. The results also showed that both Chipaya and L14 outperformed Q5 in terms of overall average grain yield and protein (%). On the other hand, the correlation coefficient can be used to enhance grain yield and protein content through other traits such as plant height, albumin (a) and albumin (b), which contributes to future plant breeding programs. HPLC analysis of the ethanol extract of the seeds of the three genotypes revealed that reveals presence of variety of flavonoids and phenolics. The extract of the three genotypes exhibited anticancer activity against hepatocellular carcinoma cell line. It is recommended to make further investigation on the biological activity of quinoa as it is a rich source of active constituents.

## Conflicting interests

The authors declare that there are no conflicts of interest that would influence the results of this work.

## Funding

This paper received no external funding.

## Tables

**Table (1):** Mean performance of some traits in tested three genotypes different genotypes of *Chenopodium quinoa* Willd. during growing season 2022/2023.

| | **Plant height (cm)** | **Chlorophyll content (SPAD)** | **Weight of 1000 grain (g)** | **Grain yield / fed. (kg)** |
|---|---|---|---|---|
| **Chipaya** | 88.0 [a] | 46.31 [b] | 3.02 [a] | 882.67 [b] |
| **L14** | 93.0 [a] | 54.20 [a] | 3.18 [a] | 905.93 [a] |
| **Q5** | 76.0 [b] | 53.98 [a] | 3.43 [a] | 822.06 [c] |
| **Mean** | 85.67 | 51.50 | 3.21 | 870.22 |
| **Standard error** | 3.68 | 1.37 | 0.11 | 4.12 |

**Table (2).** The percentage of total protein and some essential amino acids as a percentage of total protein and remaining protein in three genotypes of *Chenopodium quinoa* Willd. grains.

| | Protein (%) / grain | Albumin a (%)/ total protein (%) | Albumin b (%)/ total protein (%) | Globulin (%)/ total protein (%) | Residual (%) |
|---|---|---|---|---|---|
| **Chipaya** | 14.64 [a] | 14.07 [a] | 2.61 [a] | 51.80 [a] | 31.52 [b] |
| **L14** | 14.11 [a] | 12.57 [b] | 1.90 [b] | 53.40 [a] | 32.13 [a] |
| **Q5** | 13.40 [b] | 11.35 [c] | 1.22 [c] | 49.82 [a] | 37.61 [a] |
| **Mean** | **14.05** | **12.66** | **1.91** | **51.67** | **33.75** |
| **Standard error** | **0.21** | **0.36** | **0.06** | **1.85** | **1.97** |

**Table (3).** Correlation coefficient between the studied traits.

| **Trait** | **Plant height** | **Chlorophyll content** | **Weight 1000 grain** | **Grain yield** | **Protein (%)** | **Albumin (a)** | **Albumin (b)** |
|---|---|---|---|---|---|---|---|
| **Chlorophyll content** | -0.207[ns] | | | | | | |
| **Weight 1000 grain** | -0.773* | 0.781* | | | | | |
| **Grain yield** | 1.000* | -0.225 [ns] | -0.784* | | | | |
| **Protein (%)** | 0.745* | -0.807* | -0.999* | 0.757* | | | |
| **Albumin (a)** | 0.642* | -0.883* | -0.983* | 0.656* | 0.990* | | |
| **Albumin (b)** | 0.678* | -0.860* | -0.990* | 0.691* | 0.995* | 0.999* | |
| **Globulin** | 0.985* | -0.037 [ns] | -0.652* | 0.982* | 0.620* | 0.502* | 0.542* |

**Table (4)** Flavonoid and phenolic contents of in three genotypes of Chenopodium *quinoa* Willd. grains using HPLC.

| | **Chipaya Conc. (µg/g)** | **L14 Conc. (µg/g)** | **Q5 Conc. (µg/g)** |
|---|---|---|---|
| **Naringenin** | 46.09 | 10.04 | 3.15 |
| **Daidzein** | 88.42 | 1.06 | 6.14 |
| **Querectin** | 102.20 | 2.09 | 7.45 |
| **Kaempferol** | 24.27 | 0.00 | 0.00 |
| **Hesperetin** | 12.76 | 5.88 | 22.19 |
| **Catechin** | 60.31 | 0.00 | 0.00 |
| **Rutin** | 0.00 | 0.00 | 0.00 |
| **Gallic acid** | 22.03 | 30.74 | 77.92 |
| **Chlorogenic acid** | 0.00 | 35.73 | 78.95 |
| **Ellagic acid** | 0.00 | 0.00 | 17.17 |
| **Methyl gallate** | 8.22 | 9.51 | 25.57 |
| **Coffeic acid** | 2.43 | 8.55 | 0.00 |
| **Syringic acid** | 69.39 | 68.88 | 77.81 |
| **Coumaric acid** | 56.37 | 5.57 | 4.20 |
| **Rosmarinic acid** | 20.99 | 1.72 | 16.29 |
| **Cinnamic acid** | 12.18 | 1.31 | 4.33 |
| **Vanillin** | 98.32 | 25.34 | 5.27 |
| **Ferulic acid** | 44.71 | 4.57 | 4.01 |

**Table (5).** The One-way ANOVA results for the surviving fractions of *Chenopodium quinoa* Willd. grains extract (Chipaya, L14, Q5). *The mean difference is significant at ($P < 0.05$). Groups: Genotypes (chipaya, L14 and Q5)

| **Conc. (µg/ml)** | **Surviving fractions** | **Sum of squares** | **Degree of freedom (df)** | **Mean square** | **F-value** | **Significance** |
|---|---|---|---|---|---|---|
| 31.25 | Between groups | 0.00003467 | 2 | 0.00001734 | 1.1145 | 0.3876 |
| | Within groups | 0.00009333 | 6 | 0.00001556 | | |
| | Total | 0.000128 | 8 | | | |
| 62.5 | Between groups | 0.0005095 | 2 | 0.0002548 | 6.6462 | 0.03008* |
| | Within groups | 0.00023 | 6 | 0.00003833 | | |
| | Total | 0.0007395 | 8 | | | |
| 125 | Between groups | 0.2661 | 2 | 0.133 | 2696.5375 | 1.372e-9* |
| | Within groups | 0.000296 | 6 | 0.00004933 | | |
| | Total | 0.2664 | 8 | | | |
| 250 | Between groups | 0.0484 | 2 | 0.0242 | 105.2132 | 0.00002131* |
| | Within groups | 0.00138 | 6 | 0.00023 | | |
| | Total | 0.04978 | 8 | | | |
| 500 | Between groups | 0.0002007 | 2 | 0.0001003 | 3.0819 | 0.12 |
| | Within groups | 0.0001953 | 6 | 0.00003256 | | |
| | Total | 0.000396 | 8 | | | |
| 1000 | Between groups | 0.0006507 | 2 | 0.0003253 | 17.5328 | 0.003119* |
| | Within groups | 0.0001113 | 6 | 0.00001856 | | |
| | Total | 0.000762 | 8 | | | |

**Table (6).** The Multiple Comparison: Tukey HSD / Tukey Kramer results for the surviving fractions of *Chenopodium quinoa* Willd. grains extract. *The mean difference is significant at ($P$ < 0.05). Group 1: Chipaya, and, Group 2: L14, Group 3: Q5.

| Conc. (µg/ml) | | Significance (P-value) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 31.25 | 62.5 | 125 | 250 | 500 | 1000 |
| Group (I) versuss Group (J) | | | | | | | |
| 1 | 2 | 0.0047* | 0.0023* | 0.36 | 0.15 | 0.011* | 0.019* |
| | 3 | 0.0013* | 0.017* | 0.37 | 0.16 | 0.0077* | 0.017* |
| 2 | 1 | 0.0047* | 0.0023* | 0.36 | 0.15 | 0.011* | 0.019* |
| | 3 | 0.0033* | 0.015* | 0.008* | 0.005* | 0.0037* | 0.0013* |
| 3 | 1 | 0.0013* | 0.017* | 0.37 | 0.16 | 0.0077* | 0.017* |
| | 2 | 0.0033* | 0.015* | 0.008* | 0.005* | 0.0037* | 0.0013* |

## Figures

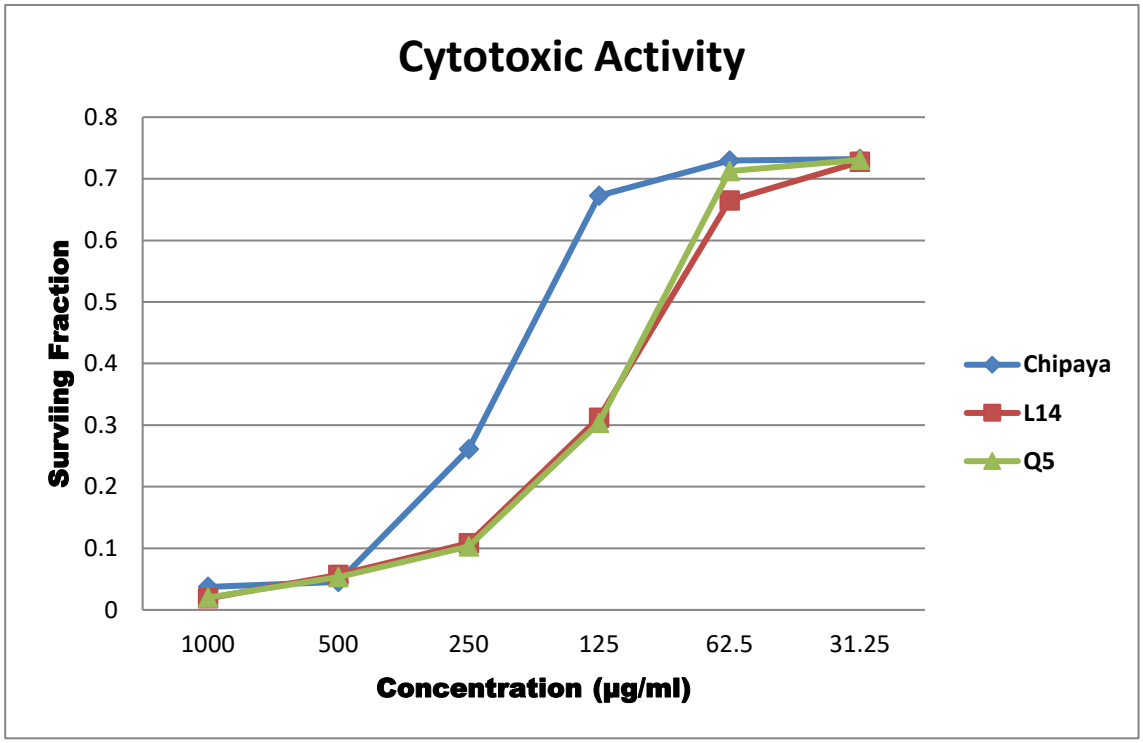


**Figure (1)** Cytotoxic activity of three genotypes of *Chenopodium quinoa* Willd. grains extract on liver carcinoma cell line (HEPG2)

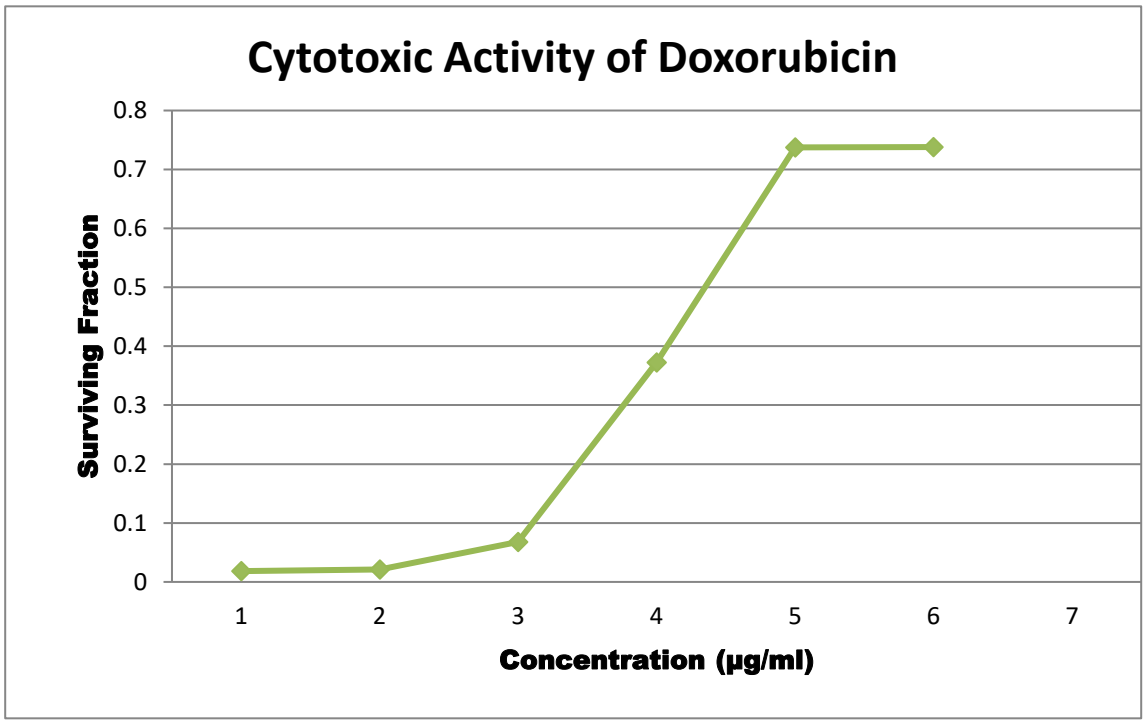


**Figure (2)** Cytotoxic activity of doxorubicin on liver carcinoma cell line (HEPG2)

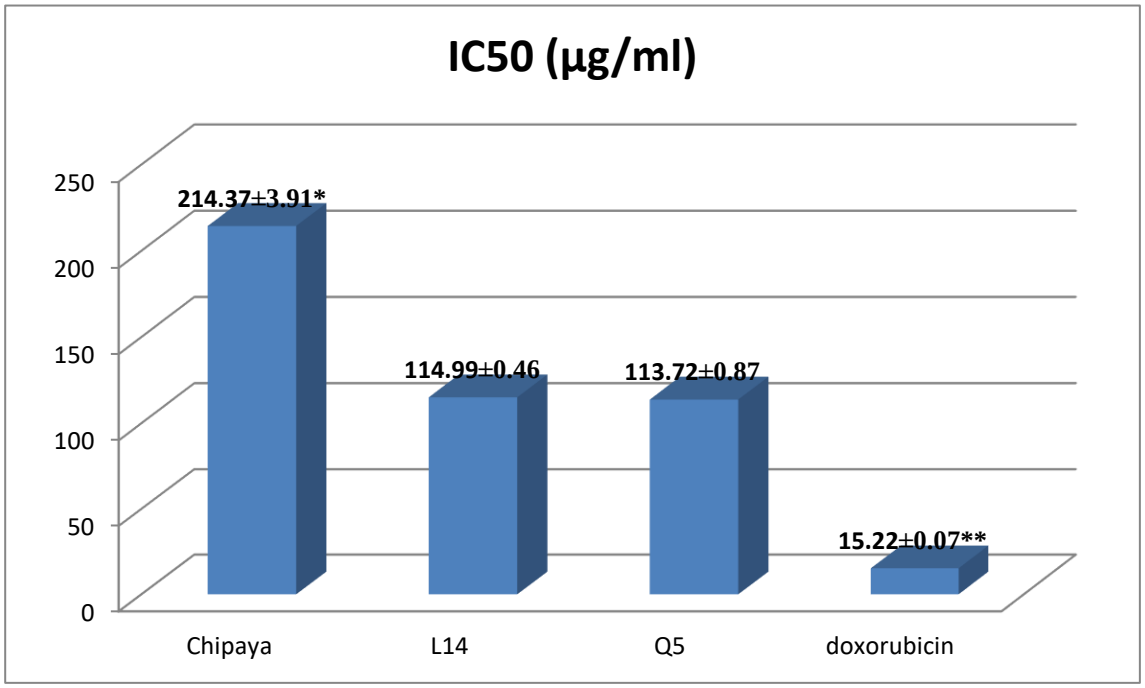


**Figure (3)** $IC_{50}$ of the three genotypes of *Chenopodium quinoa* Willd. grains extract and doxorubicin on liver carcinoma cell line (HEPG2)

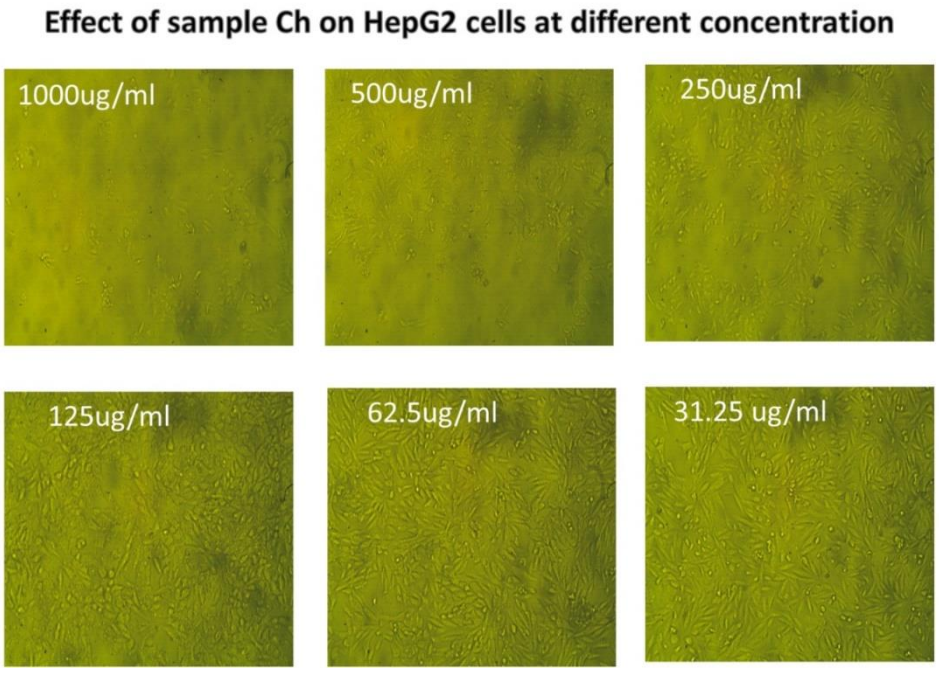


**Figure (4)** Effect of *Chenopodium quinoa* Willd. (Chipaya genotype) grains extract on liver carcinoma cell line (HEPG2)

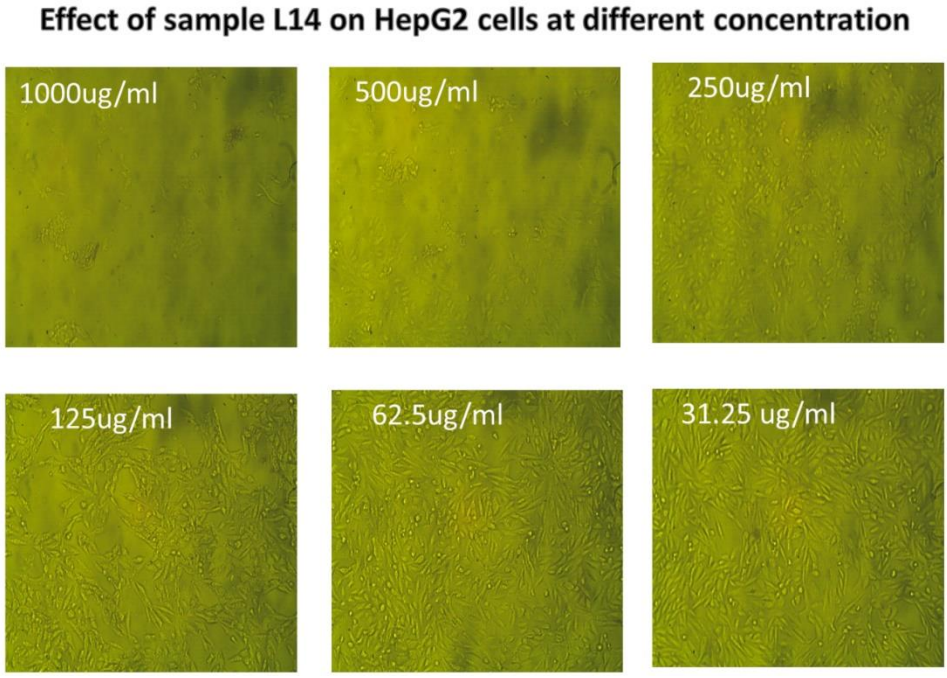


**Figure (5)** Effect of *Chenopodium quinoa* Willd. (L14 genotype) grains extract on liver carcinoma cell line (HEPG2)

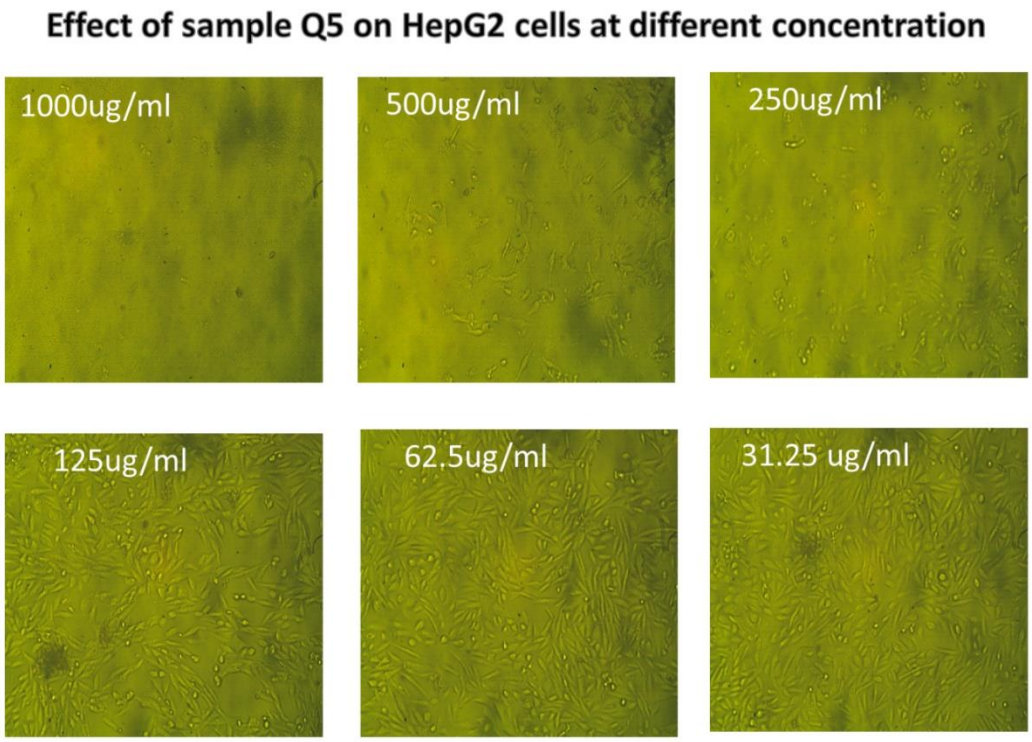


**Figure (6)** Effect of *Chenopodium quinoa* Willd. (Q5 genotype) grains extract on liver carcinoma cell line (HEPG2)

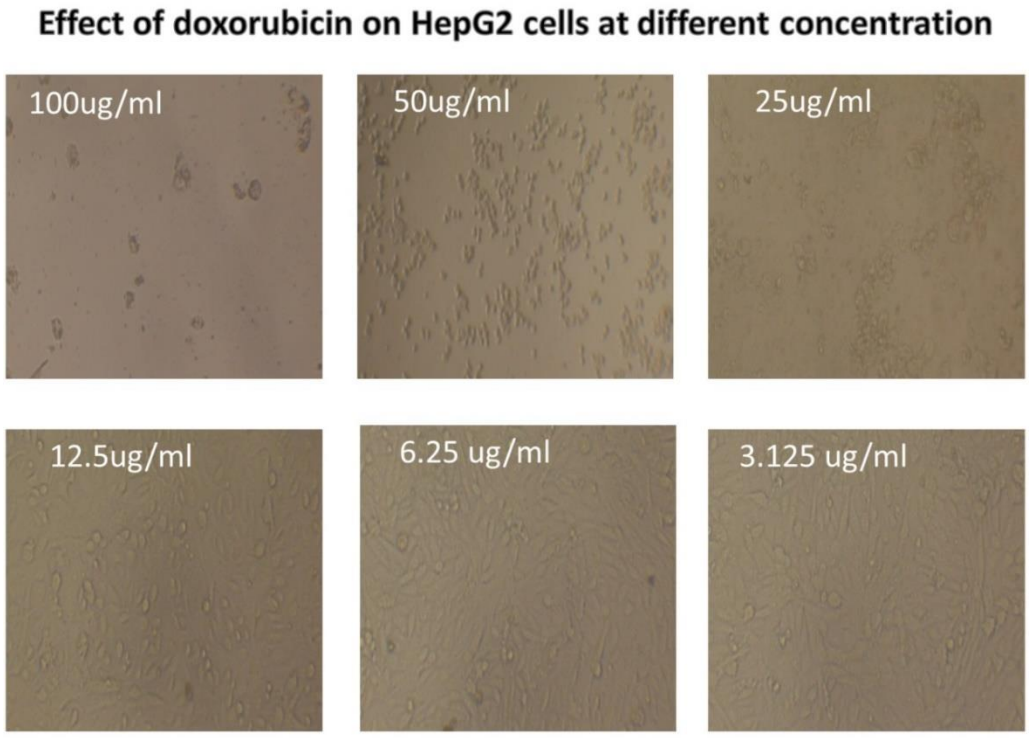


**Figure (7)** Effect of doxorubicin on liver carcinoma cell line (HEPG2)

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