Investigation of lipoidal matter of *Chenopodium quinoa* seeds and its cytotoxicity potential against three human cancer cell lines

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Background and objectives
Quinoa (*Chenopodium quinoa* Willd) is a plant species that belongs to Chenopodiaceae family. It has a high nutritional value as it is rich in proteins, lipids, and fiber, and has an extraordinary balance of essential amino acids, vitamins, and minerals, in addition to a high number of health-beneficial phytochemicals, including saponins and phytosterols. The present study aims to investigate the lipoidal components of *C. quinoa* and assessment of *C. quinoa* lipoidal components and different fractions (methanol, chloroform, ethylacetate, and butanol) cytotoxicity potential against three human cancer cell lines using MTT reduction assay.
Materials and methods
The seeds of *C. quinoa* were extracted by different solvents and the extracts prepared were tested for their lipiodial constituents and cytotoxic activities in three cancer cell lines.
Results and conclusion
The results obtained from Gas Liquid Chromatography (GLC) analysis of nonsaponifiable fraction of *C. quinoa* seeds indicated that it consists mainly of a mixture of a series of *n*-alkanes and sterols (79.96 and 7.91%. respectively). The oil was also found to be rich in linoleic acid (52.7%) and oleic acid (25.33%). The investigation of the cytotoxic potential of the plant's low polarity fractions, including $CHCl_3$, and nonsaponifiable fraction against the studied cell lines revealed the sensitivity of A549 human lung cancer cells. Further studies are needed to uncover the mechanism and chemical constituents of the obtained activity of *C. quinoa*.

Keywords:
Chenopodium quinoa, cytotoxic activity, GLC, lipid investigation

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Introduction

Chenopodium quinoa seed is an important crop that has high nutritional value owing to its high content of protein [1]. Beside its high protein content, it contains lipids, starch, minerals, fibers, and amino acids [2,3]. It also contains a plethora of phytochemicals, including saponin, phytosterols, phenolic compounds, polysaccharides, and peptides [3]. Quinoa also contains vitamin B, vitamin C, and vitamin E [4,5].

C. quinoa is an active cereal which contains many components that are important in terms of health. It has a high value of energy and nutrients, and because of its curative properties as well as the absence of gluten in it, it has benefits for consumers like children, sportsman, individuals with lactose intolerance, women with a tendency to osteoporosis, and those in a risk group such as fleshiness, celiac disease, anemia, or diabetes. The usage of *C. quinoa* in the preparation of pastries such as cakes, cookies, dietary meals, and various foods has increased its demand within the gastronomy field [6,7].

C. quinoa oil appears to be high-quality edible oil, similar in fatty acid composition to soybean oil. Palmitic acid, oleic acid, and linoleic acid are the three dominant fatty acids of quinoa oil seed [8].

C. quinoa is known to be an excellent source of natural antioxidants, and its seed extract was considered to have high anti-inflammatory activity [9].

Cancer incidence has reached an alerting status in recent years. Medicinal plants have attracted the interests of many investigators as a relatively safe

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source for anticancer agents. These interests have led to the discovery of many effective chemotherapeutic plant-derived drugs such as taxol, vincristine, and irinotecan, which are now widely used as human anticancer drugs [10–12].

In this study, we examined the lipoid constituents of *C. quinoa* seeds and assessment of cytotoxic activity of *C. quinoa* seeds lipoidal components and different fractions (methanol, chloroform, ethylacetate, and butanol) against three different types of human cancer cell lines, including lung cancer (A549), liver cancer (HepG2), and colon cancer (Caco-2).

Experimental

Plant material

The plant was cultivated in Wadi Alsheh Farm, Asyut Governorate, in agreement with the NSPO to harvest the cultivated plants. The plant was identified by Prof. Dr Kamal Zayed, Prof. of Ecology, Botany Department, Faculty of Science, Cairo University, Cairo, Egypt, to whom the authors are deeply indebted. The herbal specimen was kept at National Research Centre herbarium as a voucher specimen (#251).

Seed extraction

Two kilograms of the defatted seeds of *C. quinoa* were extracted by maceration in aqueous methanol (80%) several times until complete exhaustion.

The extracts were combined and evaporated under reduced pressure at 45°C to yield a dark brown residue (70 g). The residue was dissolved in 200 ml distilled H_2O and was successively extracted with chloroform, ethylacetate, and butanol (3×500 ml each). The obtained yields were 1.97, 0.7, and 9.45 g, respectively.

Investigation of lipid constituents of quinoa seeds

Preparation of lipid constituents [13]

The dried powdered plant material (50 g) was extracted with petroleum ether (b.r. 40–60°C) in a soxhlet apparatus. The combined petroleum ether extract was evaporated. The residue (2.5 g) was dissolved in hot acetone and kept in the refrigerator.

Saponification of acetone-soluble fraction

The acetone-soluble fraction (1.92 g) was saponified by refluxing with 100 ml N/2 alcoholic KOH for 6 h. The alcoholic solution was concentrated to ∼25 ml and diluted with cold distilled water. The unsaponifiable matter was extracted by shaking with successive portions of ether (3×50 ml). The combined ethereal extract was washed with distilled water, dehydrated over anhydrous sodium sulfate, and evaporated *in vacuo* till dryness to give 0.28 g. The unsaponifiable fraction was analyzed by Gas Liquid Chromatography (GLC).

The combined ethereal extract was washed with distilled H_2O, dehydrated over anhydrous sodium sulfate, and evaporated *in vacuo* till dryness. The unsaponifiable fraction was analyzed by GLC.

Preparation of total fatty acids

After saponification, the mother liquor was rendered acidic (pH=2) with sulfuric acid. The liberated fatty acids were thoroughly extracted several times with ether. The combined ethereal extract was washed with distilled water till free from acidity and dehydrated over anhydrous sodium sulfate. The solvent was evaporated *in vacuo* at 40°C till dryness to give 0.8 g.

GLC for unsaponifiable matter

GLC analysis of the unsaponifiable matter was carried out using Agilent Technologies 6890N series GC system. The system is equipped with a (Zb-5) capillary column (length 30 m, diameter 530 μm, and 0.5-μm film thickness).

Analysis was carried out using nitrogen as a carrier gas at a flow rate of 5 ml/min using the following temperature program: 80°C for 1 min rising at 8°C/min to 250°C. The injector temperature was 250°C, and the detector (flame ionization detector) was held at 300°C.

GLC for fatty acids as methyl esters

GLC analysis of the fatty acid methyl ester was carried out using Agilent technologies 6890N series GC system. The system is equipped with a (BPX70) capillary column (60 m length, diameter 320 μm, and 0.25-μm film thickness).

Analysis was carried out at a flow rate of 2 ml/min using the same temperature program, injector temperature, and carrier gas used for the unsaponifiable matter.

Evaluation of cytotoxic activity of different extracts and fractions

Cell culture

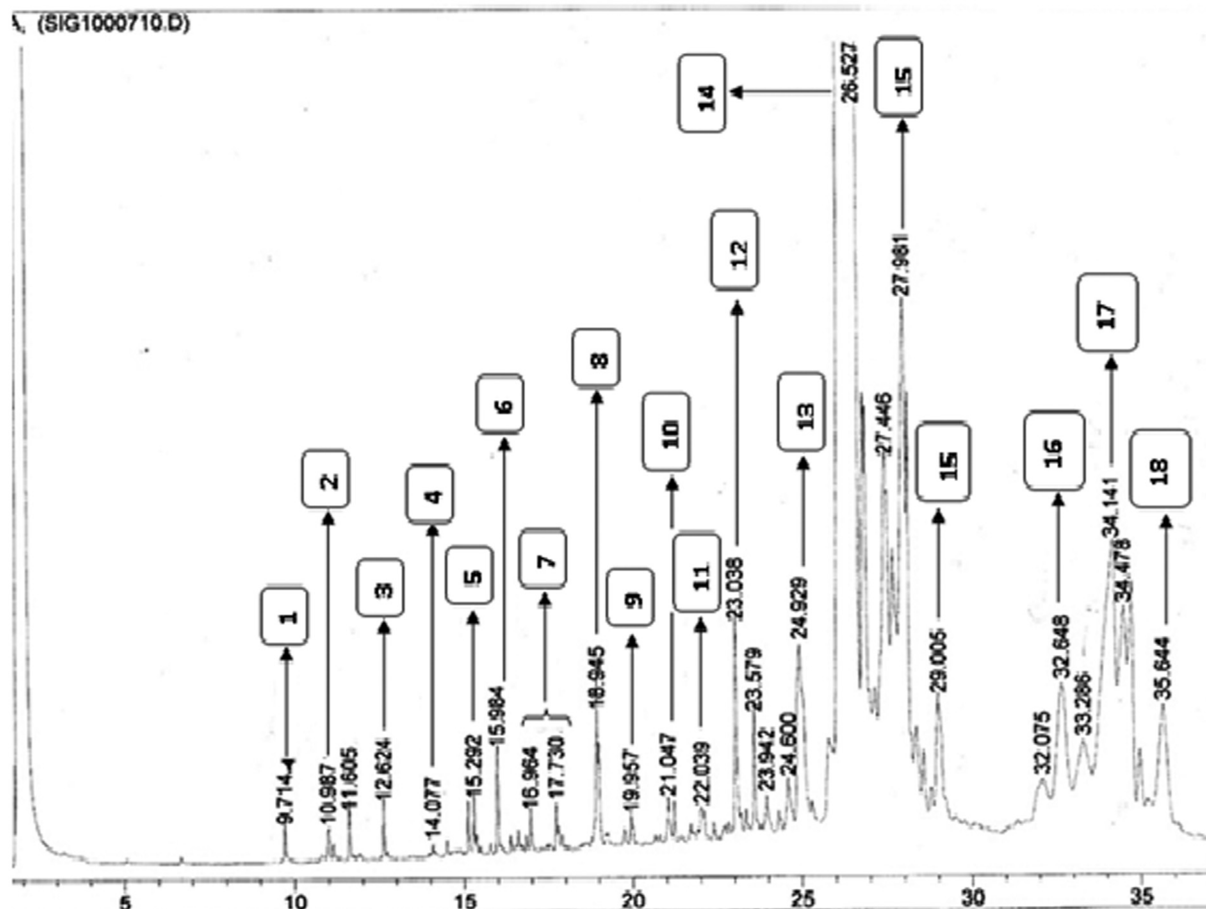
All materials and reagents for cell culture were purchased from Lonza (Belgium). Human lung cancer cell line A549 (ATCC), human cellular carcinoma cells ($HepG_2$), and human colon cancer cell line (Caco-2) were maintained as monolayer culture in Dulbecco's modified Eagle's medium

supplemented with 10% FBS, 4 mmol/l glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin sulfate. Monolayers were passaged at 70–90% confluence using trypsin-EDTA solution. All cell incubations were maintained at humidified CO_2 incubator with 5% CO_2 at 37°C.

Cytotoxicity assay

A549, $HepG_2$, or Caco-2 cells (5000–10 000 cells/well) were seeded onto 96-well plates in a total volume of 200 μl and left overnight to form a semiconfluent monolayer. We employed the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide dye (Carbosynth, UK) assay that is based on the reduction of the dye by mitochondrial dehydrogenases of metabolically active cells to insoluble formazan crystals [14]. Prescreening was performed at a single concentration of 100 μg/ml. Samples that gave more than 50% loss of cell viability were subjected to further concentration-dependent effect to determine their corresponding IC_{50}. In brief, cell monolayers were treated in rates with vehicle (DMSO, 0.1% v/v), indicated concentrations of test samples (6.25–100 μg/ml for total extracts/fractions), or doxorubicin HCl as a positive control anticancer drug for an exposure time of 48 h. At the end of exposure, MTT solution (5 mg/ml) in Dulbecco's phosphate buffered saline was added to all wells and left to incubate for 90 min at 37°C. The formation of formazan crystals was visually confirmed using phase contract microscopy. DMSO (100 μl/well) was added to dissolve the formazan crystals with shaking for 10 min after which the absorbance (OD) was read at 492 nm against the 'no cell blanks' on Sunrise microplate reader (Tecan Austria Gmbh, Grödig, Austria). Cell viability was calculated comparing the averages OD values of the control wells and those of the samples, both represented as % viability (control viability=100%). IC_{50} values (concentration of sample causing 50% loss of cell viability of the vehicle control) were calculated using the dose response curve fit to nonlinear regression correlation using GraphPad Prism, V6.0 software (San Diego, California, USA).

Figure 1



GLC analysis of unsaponifiable fractions of *Chenopodium quinoa*.

Results and discussion

Investigation of lipoidal constituents and seed oil using GLC

The results obtained from GLC analysis for unsaponifiable fraction of *C. quinoa* seeds indicated that it consists mainly of a mixture of a series of *n*-alkanes and sterols (79.96 and 7.91%, respectively). *n*-hexacosane (68.82%) and *n*-heptacosane (6.82%) were the major *n*-alkanes constituents, whereas stigmasterol (4.54%) was the major identified sterols.

Table 1 GLC analysis of unsaponifiable matter of *Chenopodium quinoa*

Number	Compound	RR_t	Area percentage
1	*n*-tridecane	0.36	0.08
2	*n*-tetradecane	0.41	0.14
3	*n*-pentadecane	0.47	0.11
4	*n*-hexadecane	0.53	0.04
5	*n*-heptadecane	0.58	0.33
6	*n*-octadecane	0.60	0.23
7	*n*-nonadecane	0.67	0.14
8	*n*-icosane	0.71	0.60
9	*n*-henicosane	0.75	0.15
10	*n*-docosane	0.8	0.21
11	*n*-tricosane	0.83	0.32
12	*n*-tetracosane	0.87	0.74
13	*n*-pentacosane	0.94	1.7
14	*n*-hexacosane	1.00	68.32
15	*n*-heptacosane	1.05	6.82
16	Campsterol	1.23	1.82
17	Stigmasterol	1.29	4.54
18	β-Sitosterol	1.34	1.55
	Total identified compounds		87.9
	Total identified hydrocarbons		79.96
	Total identified sterols		7.91
	Total unidentified compounds		12.13

RR_t, relative to *n*-hexacosane retention time=26.53 min.

GLC analysis of the fatty acids of *C. quinoa* seeds, as methyl esters, showed linoleic acid (52.7%) as the major identified fatty acid and also revealed the presence of other four fatty acids: palmitic acid (9.7%), oleic acid (25.33%), linolenic acid (8%), and arachidonic acid (1.8%).

The chromatograms and results of GLC analysis for unsaponifiable matter and fatty acids, as methyl esters, of *C. quinoa* seeds are illustrated in (Figure 1, Table 1) and (Figure 2, Table 2); respectively.

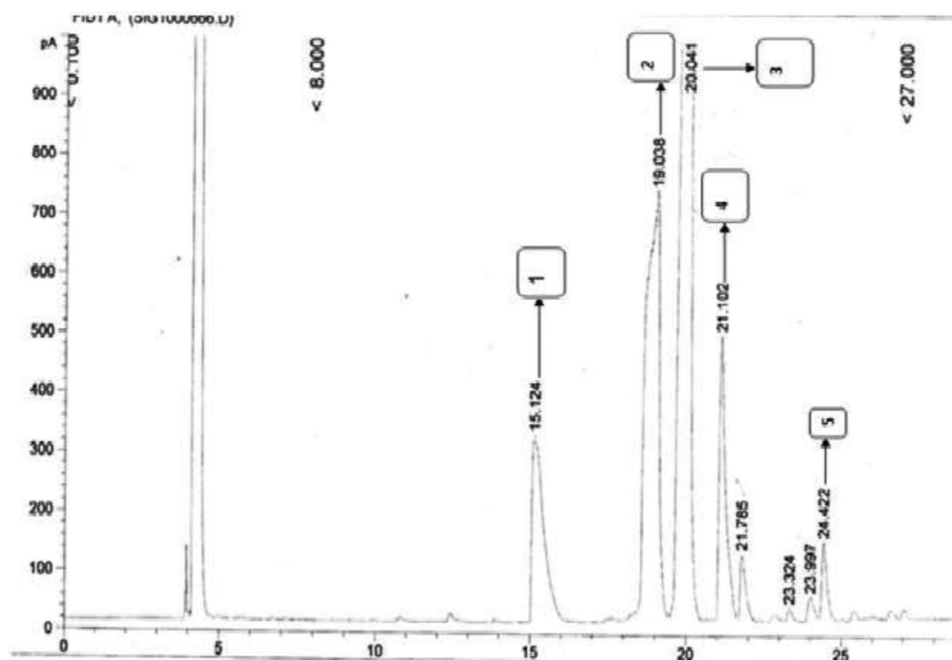
Being one of the most important crops to contribute to worldwide food security [15], and as stated in the

Table 2 GLC analysis of fatty acid as (methyl esters) of *Chenopodium quinoa*

Number	Compound	RR_t	Area percentage
1	Palmitic acid ($C_{16:0}$)	0.75	9.7
2	Oleic acid ($C_{18:1}$)	0.99	25.33
3	Linoleic acid ($C_{18:2}$)	1	52.7
4	Linolenic acid ($C_{18:3}$)	1.05	8
5	Arachidonic acid ($C_{20:4}$)	1.22	1.8
	Total identified fatty acids		97.53
	Total unidentified fatty acids		2.46

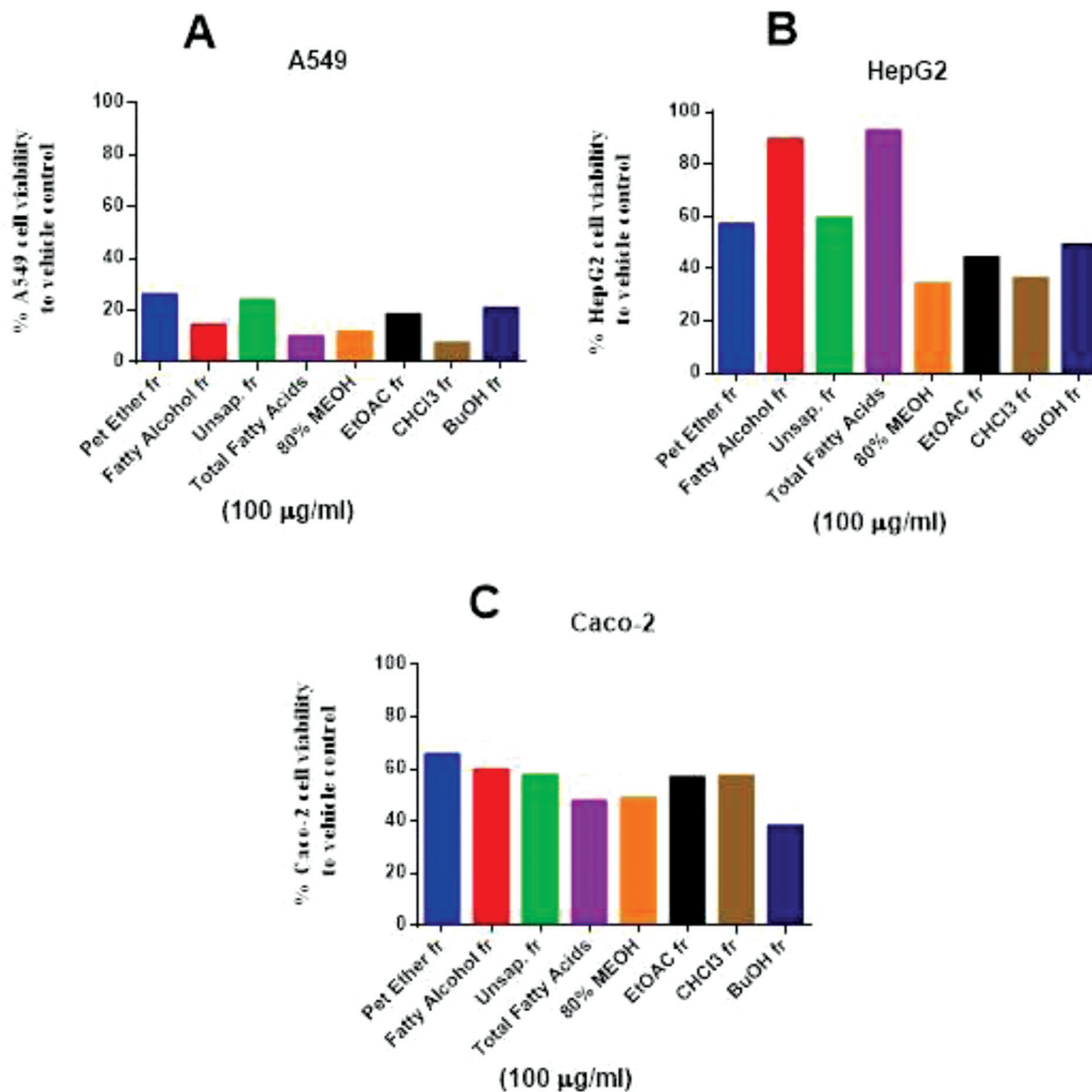
RR_t, relative to linoleic acid retention time=20.04 min.

Figure 2



GLC Analysis of fatty acid methyl ester of *Chenopodium quinoa*.

Figure 3



Pre-screening of all extracts and fractions (100 µg/ml) of *Chenopodium quinoa* against viability of three types of human cancer cell lines; lung cancer cell line A549 (A), human cellular carcinoma cells HepG2 (B) and colon cancer cell line Caco-2 (C).

literature [8], our data confirmed the high content of unsaturated fatty acid constituents in *C. quinoa* seed oil. It was found to be rich in two essential fatty acid: linolenic acid (omega-3 fatty acid) and linoleic acid (omega-6 fatty acid). As the human body is not capable of producing omega-3 fatty acid and omega-6 fatty acid, *C. quinoa* is considered as an excellent example of 'functional food,' which may help reduce the risk of various diseases.

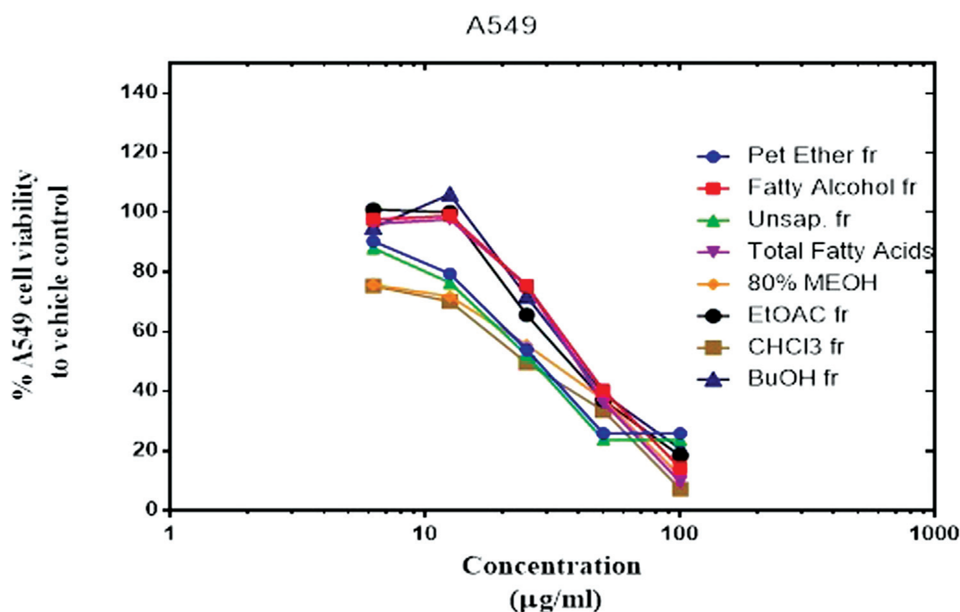
Cytotoxicity of different extracts and fractions on cancer cells

The present study aimed to evaluate different extracts and lipoid fractions of *C. quinoa* against three types of human cancer cell. As displayed in Fig. 3, the prescreen of the tested extracts and fractions revealed that human lung cancer cells A549 (Fig. 3a) were the most sensitive to treatment with single dose of 100 µg/ml. Lower response was observed against cell viability of HepG2 (Fig. 3b) and Caco-2 cells (Fig. 3c) after treatment with extracts and fractions

To determine their effective concentration causing 50% loss of cell viability (IC_{50} values), those extracts that produced considerable cytotoxic activities were further assayed for concentration-dependent effects.

Regarding human lung cancer cells (A549), treatment with extracts and fractions revealed high concentration-dependent loss of cell viability

Figure 4



Concentration-dependent effect of *Chenopodium quinoa* extracts and fractions against viability of A549 cells.

Table 3 IC_{50} values for extracts and fractions against the three studied human cancer cell lines

	IC_{50} (µg/ml)		
	A549	HepG2	Caco-2
Petroleum ether fraction	29.7	>100	>100
Fatty alcohol fraction	42.6	>100	>100
Unsaponifiable fraction	27.3	>100	>100
Total fatty acids	40.0	>100	96.4
80% MeOH	26.8	41.4	88.6
EtOAC fraction	39.3	93.9	>100
$CHCl_3$ fraction	23.1	49.8	>100
BuOH fraction	43.2	96.4	64.4

(Fig. 4). The chloroform extract caused a strong cytotoxic potential against A549 cells, recording an IC_{50} value of 23.1 µg/ml followed by the 80% MeOH extract (IC_{50}=26.8 µg/ml), unsaponifiable fraction (IC_{50}=27.3 µg/ml), and pet ether fraction (IC_{50}=29.7 µg/ml). Calculated IC_{50} values are displayed in Table 3.

As displayed in Fig. 5 and Table 3, treatment of human hepatocellular carcinoma cells ($HepG_2$) with different concentrations from *C. quinoa* extracts and fractions revealed the moderate activity by 80% MeOH extract (IC_{50}=41.4 µg/ml) and the chloroform fraction (IC_{50}=49.8 µg/ml).

Regarding human colon cancer cell (Caco-2), weaker response was produced upon treatment with increasing concentrations from the tested extracts and fractions (Fig. 6, Table 3). The BuOH fraction recorded moderate cytotoxicity potential, recording an IC_{50} value of 64.4 µg/ml.

The lipoidal components and different fractions of *C. quinoa* seeds were found to be most active as cytotoxic against lung cancer cell line A549. Bhaduri [16] has previously showed the potential of *C. quinoa* water extracts against the viability of human acute leukemia lymphocyte cell line P116. However, we presented here evidence about the potency of nonpolar, lipid-rich fractions such as chloroform and unsaponifiable fractions against lung cancer cell line A549. Further future studies are needed to uncover the identity of the involved nonpolar phytochemicals responsible for the obtained activity against cancer cells. In addition, the mechanism of the cytotoxicity potential of those active agents will further characterize the value of this super seed in the fight against cancer.

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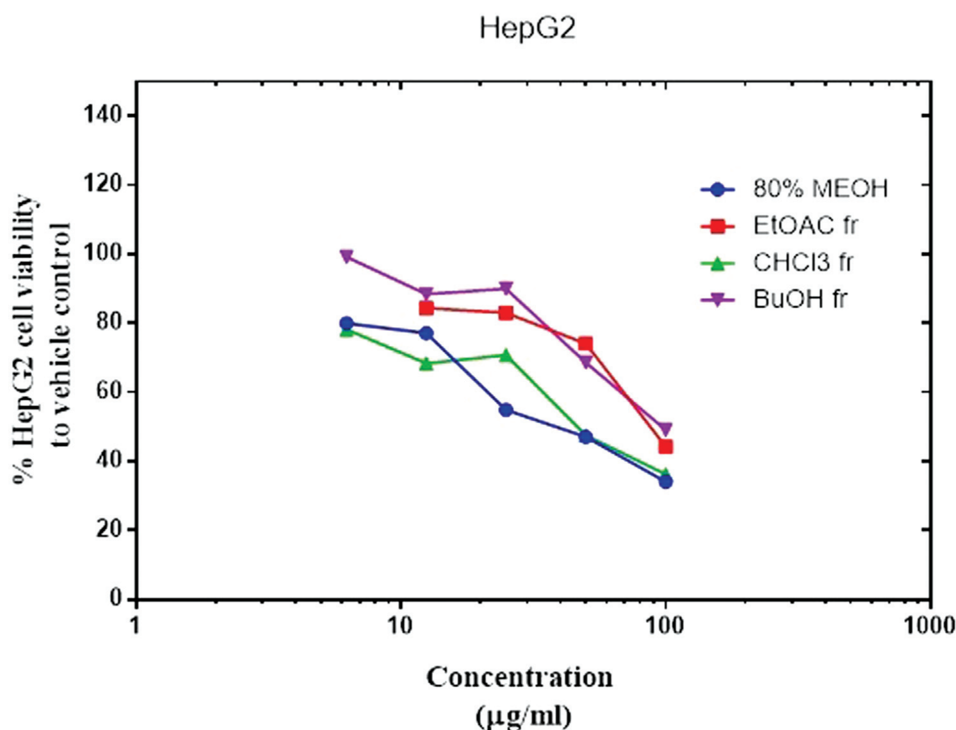
Nil.

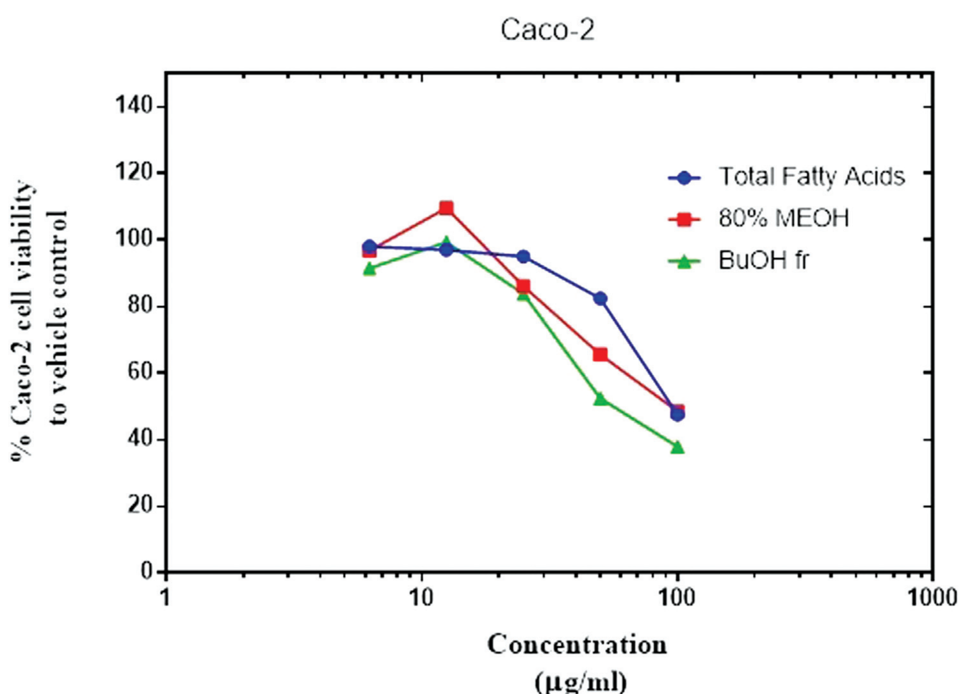
Conflicts of interest
There are no conflicts of interest.

Figure 5



Concentration-dependent effect of *Chenopodium quinoa* extracts and fractions against viability of HepG2 cells.

Figure 6



Concentration-dependent effect of extracts and fractions against Caco-2 cells.

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