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ISOLATION, PURIFICATION, AND CHARACTERIZATION OF BIOACTIVE PEPTIDE FROM CHENOPODIUM QUINOA SEEDS: THERAPEUTIC AND FUNCTIONAL INSIGHTS

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ABSTRACT

Background: *Chenopodium quinoa* is a nutrient-dense pseudocereal packed with proteins, vital amino acids, and bioactive substances that may have medicinal uses. These include antioxidant, anti-inflammatory, anti-cancer, and antibacterial properties. Notably, quinoa proteins and peptides show multifunctional bioactivities such as immunological regulation, cancer cell death, and microbial suppression. This study aimed to separate, purify, and describe the bioactive proteins found in quinoa seeds, emphasizing their potential applications as medicines. **Methodology:** Quinoa seeds underwent protein extraction, defatting, and de-saponification. Ion exchange chromatography, dialysis, and ammonium sulfate precipitation were used to purify the seeds. The Lowry technique was used to quantify the proteins. Functional tests assessed the seeds' antibacterial, antifungal, protease, and anticancer properties, and peptide identification was carried out using LC-MS/MS. **Results:** The protein content decreased during purification steps, indicating effective removal of impurities. Protein fractions exhibited significant antibacterial and antifungal activities. Protease activity varied among fractions, with the pH 2 fraction showing the highest activity. Crude extract and pH 2-treated fractions demonstrated significant anticancer activity against A549 and Hela cell lines. pH 2 fraction exhibits the highest protease activity of 2.451 units/ml, indicating enhanced enzymatic capability under acidic conditions. Peptides identified from the pH 2 fraction showed potential therapeutic properties. **Conclusion:** The antibacterial, antifungal, proteolytic, and anticancer properties of quinoa-derived peptides and proteins demonstrate their potential for use in medicine. Clinical validation and the creation of functional foods or nutraceuticals based on quinoa should be the main objectives of future research.

INTRODUCTION

Chenopodium quinoa Willd. (Quinoa), a versatile and adaptable plant with edible seeds containing proteins and some essential minerals like vitamins and dietary fiber. The protein percentage of its seed ranges between 12% and 23%; hence, it is called a significant protein source [1]. Quinoa seeds have several health

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benefits, including beneficial effects on immunity regulation, cardiovascular diseases, diabetes, inflammatory disease, and obesity [2-4]. Quinoa has vitamins, carotenoids, and tocopherols that aid in its high antioxidant activity [5]. Quinoa has several secondary metabolites in it, including flavonoids, phenolics, alkaloids, and polysaccharides. An isolated novel polysaccharide demonstrated anticancer activity on SMMC7721 (human liver cancer cell lines) and MCF-7 (Breast cancer cell line) [6]. Quinoa proteins and peptides of more than 5kDa molecular weight are found to have anticancer activity against colon cancer cell lines [7]. Quinoa seeds have omega fatty acids and a low glycaemic index and are thus considered a good food for diabetes consumption [8]. The quinoa seed powder has testified to hypolipidemic activity and is a hepatic and nephron-protector [9]. Quinoa has anti-inflammatory activity, decreasing inflammatory mRNA levels and increasing macrophage proliferation [10]. Quinoa has antibacterial activity against several pathogenic bacteria, including *Escherichia coli* (0.83-0.148 cm inhibition zone) and *Staphylococcus aureus* (0.85-0.15 cm inhibition zone) [11]. Quinoa offers an excellent supply of protein, possessing necessary amino acids. Albumin and globulin are the main proteins of quinoa, with varying concentrations in different varieties of plants [12]. Chenopodin of 316 kDa is another main protein isolated from *Chenopodium quinoa* [13]. Protein hydrolysates of quinoa have antioxidant activity and antidiabetic activity by inhibiting dipeptidyl peptidase IV (DPPIV) activity [14]. 17 protein fractions were isolated from quinoa seed; protein fractions less than 5kDa showed antioxidant activity, while fractions of more than 5kDa exhibited anticancer activity [7]. *Chenopodium quinoa* protein exhibits various beneficial properties, including antibacterial, anticancer, and protease activity, making it a valuable and versatile component with potential applications in various fields. To understand their purported health benefits, it is necessary to research the bioactive potential of protein fractions and peptides of *Chenopodium quinoa* seeds regardless of difficulties in identifying functional mechanisms. This research explicitly targets this by focusing on isolating proteins, identifying them separately, evaluating their bioactive potentials, and investigating their effects on living organisms.

MATERIALS AND METHODS

Materials: *Chenopodium quinoa* seeds, obtained from the Dryland Farming Centre in Bhilwara and certified by the same institute, were used with cell lines for scientific research. The seeds were stored in an airtight glass container at room temperature. The L132 cell line, representing normal lung cells, and two cancer cell lines—A549 (lung cancer) and HeLa (cervical cancer)—were obtained from the National Centre for Cell Science (NCCS), Pune. Statistical analysis of the obtained data was done using SPSS software.

Methods

Crude processing

Quinoa seeds were de-saponified and defatted after washing [15], shade-drying, and grinding into powder. The quinoa was defatted with chloroform and methanol solvent (2:1). Three runs of this step were made. The defatted flour was then mixed with distilled water, pH altered to 10.0, and incubated for an hour with subsequent centrifugation for 30 minutes at 5000 rpm. The supernatant was placed in a fresh tube.

Ammonium sulphate precipitation

The quinoa protein was sequentially subjected to ammonium sulphate precipitation, starting with 10%, followed by 20%, and finally reaching 30%. This process involved overnight incubation at 4°C, then centrifugation for ten minutes at 10,000 rpm after each step. Protein was estimated for each fraction. Fractions with high protein content, anticancer, and antimicrobial activity were dialyzed and purified based on their pI.

Quinoa protein separation based on their pI

A 10 ml aliquot of ammonium sulphate precipitate underwent a pH adjustment to 2, followed by centrifugation for ten minutes at 12,000 rpm. The resultant pellet was reconstituted in 5 ml of deionized (DI) water. The supernatant's pH was adjusted after a 10-minute centrifugation at 12,000 rpm. This stepwise procedure was repeated for various protein fractions, each precipitated at specific pH values (6, 8, 9, 10, 11, 12, 14). Protein content was quantified, and each fraction was assessed for anticancer and antimicrobial activity. Fractions demonstrating significant activity were further processed through ammonium sulphate fractionation.

Protein purification using Ion exchange chromatography

The protein fractions were subjected to additional purification [16], employing a DEAE cellulose column (2X18 cm) pre-equilibrated with a 50 mM Tris-HCl buffer at a pH of 8.0. Protein elution was achieved using the same buffer

supplemented with incremental concentrations of NaCl (0.1M, 0.5M, 1M, 1.5M, 2.0M, and 2.5M). Fractions devoid of protein were discarded, as determined by a lack of absorbance at 280 nm in UV spectrophotometry. The remaining fractions were subsequently evaluated for their potential anticancer and antibacterial activities.

Protein estimation using the Lowry method

Protein was estimated for crude and all the purified fractions following the protocol of Lowry [17]. Briefly, 4.5 ml of reagent I (48 ml of solution A (2% Sodium carbonate prepared in 0.1N NaOH: 1 ml of 0.5% Copper Sulphate: 1 ml of 1% Sodium potassium tartrate) was mixed with 0.1 ml of crude and 0.9 ml of distilled water. After thoroughly mixing and allowing the mixture to settle at room temperature for 10 minutes, 0.5 milliliters of 1N Folin-Ciocalteu (water: FC reagent; 1:1) was added. The mixture was mixed properly, followed by half-hour incubation at room temperature. Absorbance was taken at 650 nm against a blank of all reagents except the standard or sample. The bovine serum (BSA) standard graph was prepared by taking absorbances for different dilutions of BSA. Protein was estimated from the standard curve equation obtained by plotting the graph between BSA concentrations ranging from 10 microgram/ml to 100 microgram/ml.

Protease activity

Protease activity of quinoa crude and fractions measured by the method described by Cupp-Enyard [18]. In the protease activity assay, a Tyrosine Standard Curve determines the concentration of µmoles of tyrosine equivalents liberated. The curve is generated by diluting a stock solution of L-Tyrosine (1.1 mM) to various concentrations, ranging from 5.5 µM to 275 µM. During the assay, 100 µl of the crude and fractions were mixed with 1.4 ml acetate buffer (0.2M, pH 5.0) and 2 ml of a 1% haemoglobin solution in acetate buffer (0.1M, pH 5.0). After 30 minutes of incubation at 37°C, the reaction is halted by mixing 1 ml of 10% TCA and incubating for an additional 10 minutes.

After centrifugation, 1 ml of the supernatant is collected and mixed with 1 ml of sodium carbonate solution and 1 ml of FC reagent. The resultant mixture is incubated at room temperature for thirty minutes, and the absorbance is measured at 660 nm. The amount of tyrosine released is calculated by referring to the Tyrosine Standard Curve. The amount of an enzyme that produces one µmol of tyrosine per minute at 37°C is known as protease activity. The specific activity is measured in units per milligram (Unit/mg).

Anticancer activity and IC_{50} estimation of quinoa crude protein

Cytotoxic activity was estimated by the method of Mossman, 1983 [19]. Briefly, crude and fractions were diluted in the 1 to 40 µg/ml range. The samples were combined with the cell lines cultured for a full day in a confluent monolayer plate. Following a 72-hour incubation at 37°C in an incubator with 5% CO_2, the supernatant was removed, and each well received 25 µl of MTT reagent (2 mg/ml). Following a 4-hour incubation period at 37°C, each well received 100 µl of dimethyl sulfoxide. The wells were shaken for 15 minutes to dissolve the formazan precipitate. An ELISA reader was used to calculate the absorbance at 490 nm. Alone medium, DMSO, and cells are negative controls without the examined chemical.

$$\%\ Cell\ cytotoxicity = \left(\frac{A0 - At}{A0}\right) * 100$$

where;

A0 is the absorbance of the control and

A1 is the absorbance of the extract and fractions.

Preparation of protein hydrolysate and peptides

All the protein fractions with potential anticancer activity were proceeded for hydrolysis [20]. pH of fractions was adjusted to 2.0 by adding 1M HCl. Trypsin (5%) solution was mixed with the fractions and incubated for 1 hr. at 37°C, then increasing the pH to 7.0. Pancreatin (5%) was added to the hydrolysate and incubated for 1 hr. at 37^0C. Hydrolysates were boiled for 5 min and cooled down before centrifugation at 5000 rpm at 4^0C for 20 min. Suspension was collected and proceeded for filtration through a 5kDa ultrafiltration membrane. Fractions of more than 5 kDa were collected and stored. The highest antiproliferative activity fractions were proceeded for MW by LC-Tandem mass spectrophotometer (LC-MS/MS).

Identification of peptides

Peptides were identified by searching the MW of peptides against the protein database in NCBI.

Statistical analysis

IBM SPSS Statistics (version XX) was used for all statistical analyses. The software carried out descriptive statistics, hypothesis testing, and data validation. One-way ANOVA was

used for group comparisons, and post hoc tests (such as Tukey's or Bonferroni, depending on variance homogeneity) were used. The statistical significance threshold was set at $p<0.05$, and the results were presented as mean ± standard deviation. This method supported data interpretation and inference by guaranteeing the findings' robustness and dependability.

RESULTS AND DISCUSSION

Protein estimation

The crude extract's initial protein content was 1.10 mg/gm. Subsequent purification steps involved varying pH levels and salt concentrations. At pH 10, the protein content decreased to 0.31 mg/gm, indicating a significant reduction in protein concentration. These decreases continued with lower pH levels, reaching 0.11 mg/gm at pH 2. Similarly, exposure to 2M NaCl resulted in a protein content of 0.13 mg/gm (Figure 1).

The observed decrease in protein content from the crude to the subsequent purification steps suggests the effective removal of impurities and non-protein components. The decline in protein content with decreasing pH values implies that specific proteins may precipitate or become less soluble under acidic conditions. These findings are consistent with previous research on protein extraction from quinoa seeds [15].

Antimicrobial assay

The study established the efficacy of quinoa crude extract and pH 2 protein isolates against *Pseudomonas aeruginosa*. Additionally, quinoa crude extract, protein isolates at pH 2, and ion fractions 1.5M and 2.0M demonstrated significant antifungal activity against *Aspergillus nidulans* and *Aspergillus niger* (Figure 2 & Table 1).

These results align with previous investigations by [21,22], highlighting the antifungal properties of quinoa extracts and protein fractions across diverse fungal pathogens. The observed inhibitory effects on fungal growth are likely attributed to the bioactive protein present in quinoa.

Protease activity

pH 2 fraction exhibits the highest protease activity of 2.451 units/ml, indicating enhanced enzymatic capability under acidic conditions. Among ion fractions, 0.5M NaCl displays relatively high protease activity of 2.996 units/ml. In contrast, higher NaCl concentration fractions showed reduced activity, except for 2.5M NaCl, which shows a significant increase (4.948 units/ml) (Figure 3). These results highlight the influence of pH and NaCl concentration on protease activity, providing insights for applications in industries like food processing and biotechnology.

Protease activity in crude extract or protein fractions can be advantageous for various purposes. It can aid in protein hydrolysis, leading to the production of bioactive peptides with potential health benefits. Additionally, protease activity can enhance the digestibility of quinoa proteins, making them more accessible and nutritious for consumption.

Table 1: Zone of inhibition of crude, pH2 protein isolate, and ion fractions

Protein isolates	*P. aeruginosa*	*A. nidulans*	*A. Niger*
	Zone of inhibition		
C (Crude)	-	11 mm	13 mm
pH (2.0)	-	14 mm	10 mm
1.5 M NaCl	14 mm	12 mm	10 mm
2.0 M NaCl	9 mm	9 mm	15 mm

Anticancer activity and IC_{50} estimation of quinoa crude protein and fractions

Three cell lines were used to assess quinoa crude and all of its fractions: L132 (normal), Hela (cervical cancer), and A549 (lung cancer). It was shown that a normal cell line was cytotoxic to the 0.1 M NaCl fraction. L132 was shown to be non-toxic to crude extract, and its viability ranged from 52.66% to 86.59%. There is a wide variation in the pH2-treated sample (11.21% to 117.66%).

When tested against A549 cell lines, crude extract exhibits moderate to low vitality, ranging from 19.45% to 52.04%; a sample treated with pH 2 consistently displays poor viability, ranging from 11.21% to 19.28%. The viability of crude extract varies from 33.56% to 67.51% against Hela cells, whereas samples treated with pH 2 have a moderate vitality of 25.90% to 58.97% (Figure 4).

Compared to the pure extract, the pH 2-treated samples exhibit a broader range of viability overall, indicating a more varied response in several cell lines. The IC_{50} values show the crude extract is most effective in reducing cell viability for both A549

and Hela cell lines, with pH 2 treatment also showing good potency. NaCl treatments have higher IC_{50} values, indicating lower efficacy in inhibiting cell viability, especially at higher concentrations (Figure 5). Mollaei also reported a similar trend, where quinoa seed extract prevented the growth of A549 cells compared to the control [24,25].

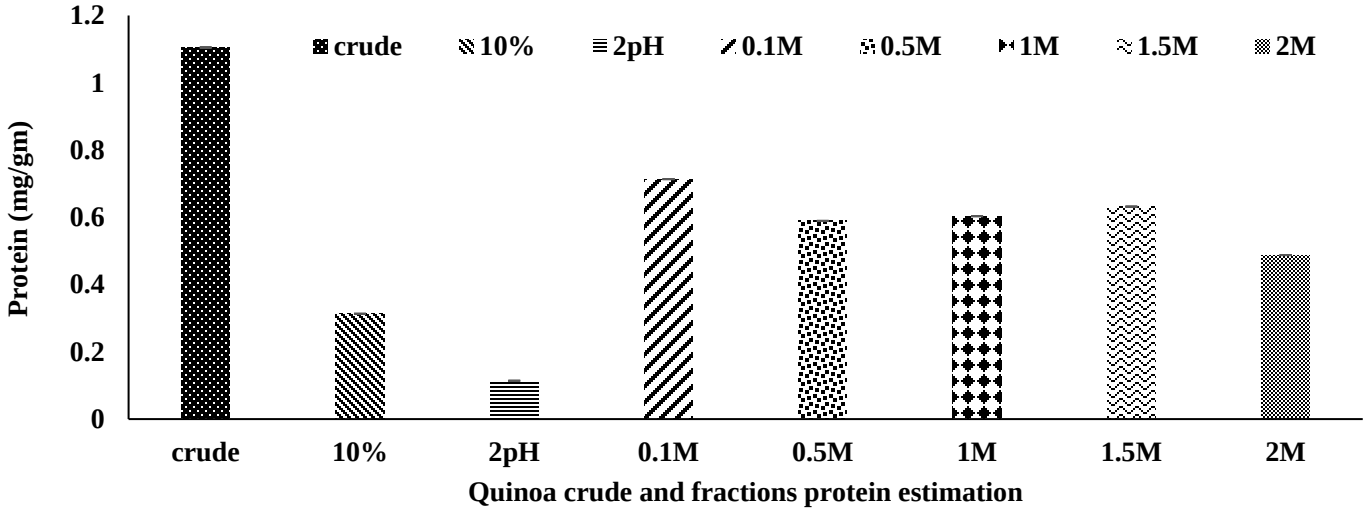

Figure 1: Protein estimation of crude, Ammonium sulphate, and ion chromatography fractions having potent anticancer and antimicrobial activity. All data are represented as triplicates.

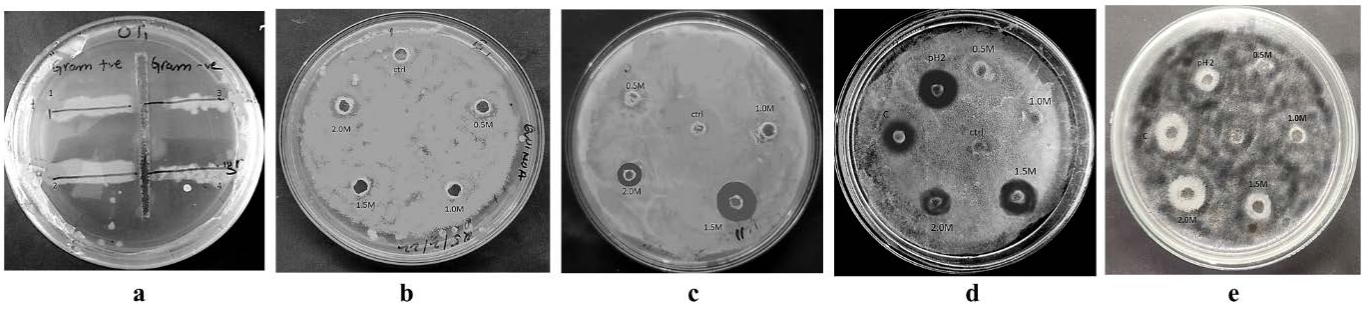

Figure 2: A: Antimicrobial activity of protein isolates. a: pH 2 protein isolate with 1: *N. bataviensis,* 2: *L. acetophenone,* 3: *P. aeruginosa,* 4: *S. pavanii,* b: ion fractions against *S. pavanii,* c*:* against *P. aeruginosa,* d: activity of crude, pH 2 and ion fractions against *A. nidulans,* e: crude, pH 2 and ion fractions against *A. niger.*

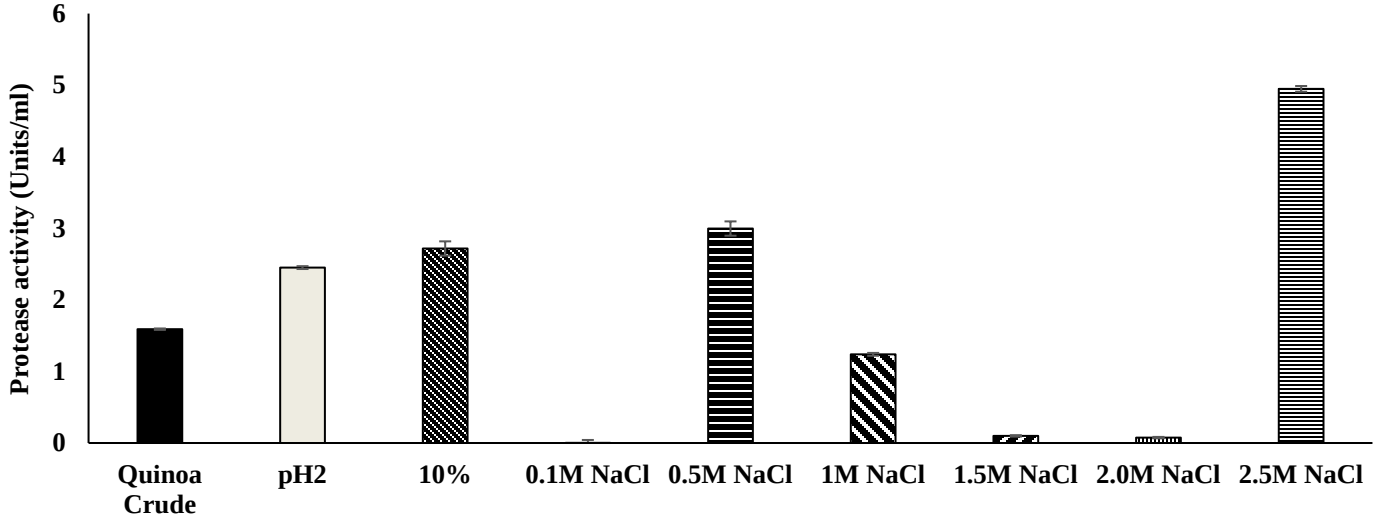

Figure 3: Protease activity (Units/ml) of crude, pH protein isolates and ion fractions. All data are represented as triplicates

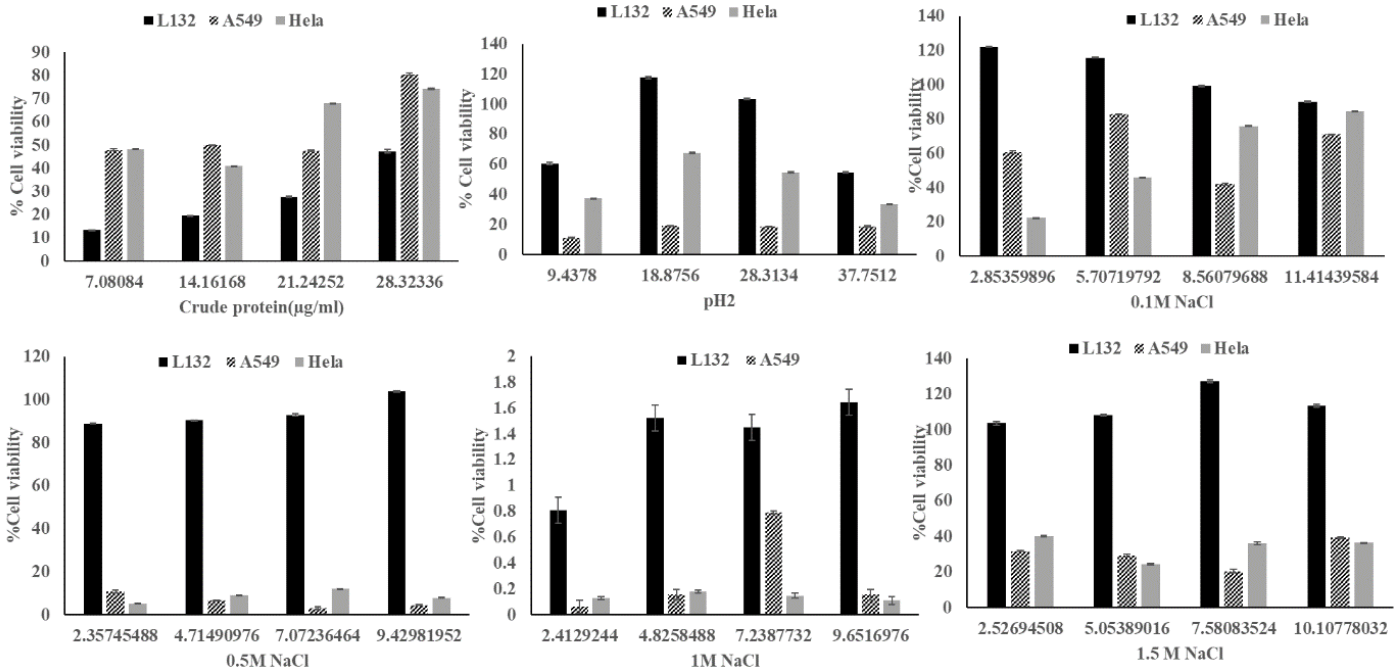

Figure 4: % cell viability of crude and various fractions at different dilutions against L132, A549, and Hela cell lines

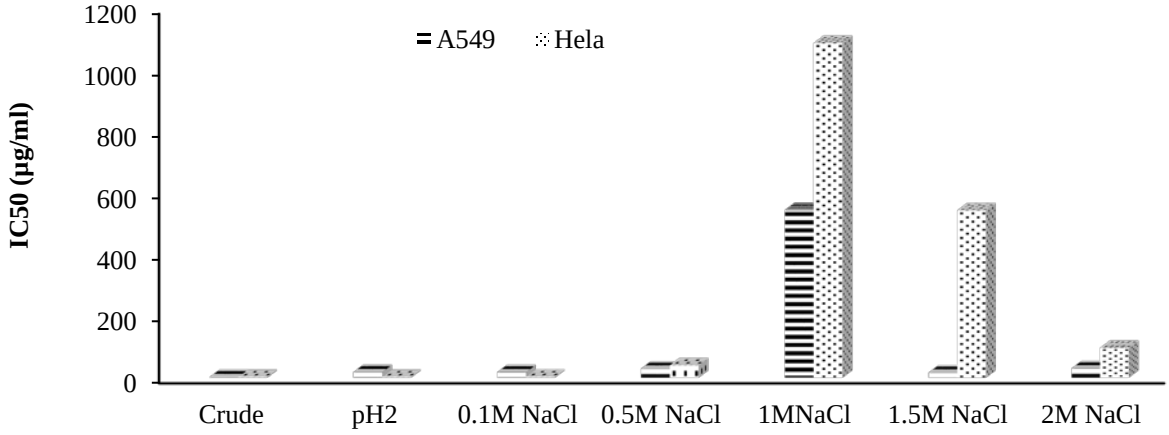

Figure 5: Half maximal inhibitory concentration of crude and all the fractions against A549 and Hela cell lines.

Peptide sequencing

The most therapeutic-potent fractions were pH 2, 10%, and 2M. The molecular weight of each fraction was calculated using LC-MS/MS. Only the pH2 fraction, with an MW of 14169.47 Da (Figure 7), was found in the NCBI protein database with the following details. uncharacterized protein LOC110740183 [*Chenopodium quinoa*] (Accession number: XP_021776352) with protein sequence of >XP_021776352.1 uncharacterized protein LOC110740183 [*Chenopodium quinoa*]

MEKKKSTKLGASNRQFHTDETRIPDYERSRLQRIRENVL KMQALGLHEKAMSLMGRYQTEKGSRGKINKKKDAEED EIYRPDQDSDIDEHEHDSSFEGTRIKETLSNQGSNSNRKK VITSNCN

CONCLUSION

The study successfully extracted and described several bioactive proteins from quinoa seeds, exhibiting antibacterial, antifungal, proteolytic, and anti-neoplastic qualities. These results demonstrate the potential medicinal and commercial uses of proteins obtained from quinoa, especially in the biomedical domains for drug discovery and in manufacturing functional foods and nutraceuticals.

Future studies should concentrate on focused bioassays to confirm the bioactivity of these peptides and further clarify the processes underlying their functional characteristics to expand on these encouraging findings. Clinical evaluations and sophisticated structural research are also advised to investigate their potential for medicinal and commercial uses.

FINANCIAL ASSISTANCE
NIL

CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTION
Amit Sen, Gunjan Sharma, and Nalini Tomer conducted laboratory experiments and statistical analysis. Sahaya Shibu B. interpreted the results. Sarmad Moin conceptualized, planned, and executed the research and oversaw the final decisions.

REFERENCES
[1] Yang C, Zhu X, Zhang Z, Yang F, Wei Y, Zhang F. Yang. Heat treatment of quinoa (*Chenopodium quinoa* Willd.) albumin: Effect on structural, functional, and in vitro digestion properties. *Front. Nutr.*, **9,** (2022) https://doi.org/10.3389/fnut.2022.1010617

[2] Graf BL, Rojas-Silva P, Rojo LE, Delatorre-Herrera L, Baldeón ME, Raskin I. Innovations in Health Value and Functional Food Development of Quinoa (Chenopodium quinoa Willd.). *Compr. Rev. Food Sci. Food Saf.*, **14**(4), 431–445 (2015) https://doi.org/10.1111/1541-4337.12135

[3] Navruz-Varli S, Sanlier N. Nutritional and health benefits of quinoa (Chenopodium quinoa Willd.). *J. Cereal Sci.*, **69**, 371–376 (2016) https://doi.org/10.1016/j.jcs.2016.05.004

[4] Yao Y, Shi Z, Ren G. Antioxidant and Immunoregulatory Activity of Polysaccharides from Quinoa (*Chenopodium quinoa* Willd.). *Int. J. Mol. Sc.*, **15**(10), 19307–19318 (2014) https://doi.org/10.3390/ijms151019307

[5] Tang Y, Li X, Chen PX, Zhang B, Hernandez M, Zhang H, Marcone MF, Liu R, Tsao R. Characterisation of fatty acid, carotenoid, tocopherol/tocotrienol compositions and antioxidant activities in seeds of three Chenopodium quinoa Willd. genotypes. *Food Chem.*, **174**, 502–508 (2015) https://doi.org/10.1016/j.foodchem.2014.11.040

[6] Hu Y, Zhang J, Zou L, Fu C, Li P, Zhao G. Chemical characterization, antioxidant, immune-regulating and anticancer activities of a novel bioactive polysaccharide from Chenopodium quinoa seeds. *Int. J. Biol. Macromol.*, **99**, 622–629 (2017) https://doi.org/10.1016/j.ijbiomac.2017.03.019

[7] Vilcacundo R, Miralles B, Carrillo W, Hernández-Ledesma B. In vitro chemopreventive properties of peptides released from quinoa (*Chenopodium quinoa* Willd.) protein under simulated gastrointestinal digestion. *Food Res. Int.*, **105**, 403–411 (2018) https://doi.org/10.1016/j.foodres.2017.11.036

[8] Priyanka M, Suneetha K, Maheswari K, Suneetha B, Kumari B. Glycemic Index Profiling of Quinoa (Chenopodium quinoa Willd) Variety. *J. Pharm. Res. Int.*, **21**(2), 1–5 (2018) https://doi.org/10.9734/jpri/2018/37915

[9] Ng CY & Wang M. The functional ingredients of quinoa (*Chenopodium quinoa*) and physiological effects of consuming quinoa: A review. *Food Front.*, **2**, 329–356 (2021) https://doi.org/10.1002/fft2.109

[10] Foucault A, Mathé V, Lafont R, Even P, Dioh W, Veillet S, Tomé D, Huneau J, Hermier D, Quignard-Boulangé A. Quinoa Extract Enriched in 20-Hydroxyecdysone Protects Mice from Diet-Induced Obesity and Modulates Adipokines Expression. *Obes.*, **20**(2), 270–277 (2012) https://doi.org/10.1038/oby.2011.257

[11] Miranda M, Delatorre-Herrera J, Vega-Gálvez A, Jorquera E, Quispe-Fuentes I, Martínez EA. Antimicrobial Potential and Phytochemical Content of Six Diverse Sources of Quinoa Seeds (*Chenopodium quinoa* Willd.). *Agric. Sci.*, **05**(11), 1015–1024 (2014) https://doi.org/10.4236/as.2014.511110

[12] Abugoch LE. Chapter1 Quinoa (Chenopodium quinoa Willd.): Composition, Chemistry, Nutritional and Functional Properties. *Adv. Food Nutr. Res.*, **58**, 1–31 (2009) https://doi.org/10.1016/s1043-4526(09)58001-1

[13] Brinegar C, Saraswathi G. Isolation and characterization of chenopodin, the 11S seed storage protein of quinoa (*Chenopodium quinoa*). *J. Agric. Food Chem.*, **41**(2), 182–185 (1993) https://doi.org/10.1021/jf00026a006

[14] Nongonierma AB, Maux S, Dubrulle C, Barre C, FitzGerald RJ. Quinoa (Chenopodium quinoa Willd.) protein hydrolysates with in vitro dipeptidyl peptidase IV (DPP-IV) inhibitory and antioxidant properties. *J. Cereal Sci.*, **65**, 112–118 (2015) https://doi.org/10.1016/j.jcs.2015.07.004

[15] Elsohaimy SA, Refaay TM, Zaytoun MAM. Physicochemical and functional properties of quinoa protein isolate. *Ann. Agric. Sci*., **60**(2), 297–305 (2015) https://doi.org/10.1016/j.aoas.2015.10.007

[16] Capraro JS, De Benedetti M, Di Dio E, Bona A, Abate A, Corsetto A, Scarafoni A. Characterization of Chenopodin Isoforms from Quinoa Seeds and Assessment of Their Potential Anti-Inflammatory Activity in Caco-2 Cells. *Biomol.*, **10**(5), 795 (2020) https://doi.org/10.3390/biom10050795

[17] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193**(1), 265–275 (1951) https://doi.org/10.1016/s0021-9258(19)52451-6

[18] Cupp-Enyard C. Sigma's Non-specific Protease Activity Assay - Casein as a Substrate. *J. Vis. Exp.*, 19 (2008) https://doi.org/10.3791/899

[19] Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, **65**(1–2), 55–63 (1983) https://doi.org/10.1016/0022-1759(83)90303-4

[20] Guo Y, Shen R, Yu L, Zheng X, Cui R, Song Y, Wang D. Roles of galectin-3 in the tumor microenvironment and tumor metabolism (Review). *Oncol. Rep.* (2020) https://doi.org/10.3892/or.2020.7777

[21] Ragucci S, Daniela B, Nicola L, Rosita R, Angela C, Mariangela V, Emanuela G, Franco F, Antimo DM. The structural characterization and antipathogenic activities of quinoa, a type 1 ribosome-inactivating protein from quinoa seeds. *Int. J. Mol. Sc.*, **22**(16), 8964 (2021) https://doi.org/10.3390/ijms22168964

[22] Stuardo M, San Martín R. Antifungal properties of quinoa (Chenopodium quinoa Willd) alkali-treated saponins against Botrytis cinerea. *Ind. Crops. Prod.*, **27**(3), 296-302 (2008) https://doi.org/10.1016/j.indcrop.2007.11.003

[23] Mudgil P, Omar LS, Kamal H, Kilari BP Maqsood S. Multi-functional bioactive properties of intact and enzymatically hydrolyzed quinoa and amaranth proteins. *LWT*, **110**, 207–213 (2019) https://doi.org/10.1016/j.lwt.2019.04.084

[24] Mollaei HF, Karimi M, Ghorbany MS, Hosseinzadeh M, Moudi M, Mousavi-Kouhi SM. Investigating cytotoxic effect and molecular mechanisms of quinoa seed extract against human lung cancer cell line. *Jentashapir J. Cell. Mol. Biol.*, 1**2**(4) (2021) https://doi.org/10.5812/jjcmb.121089

[25] Aluko RE, Monu E.Functional and Bioactive Properties of Quinoa Seed Protein Hydrolysates. *J. Food Sci.*, **68**(4), 1254–1258 (2003) https://doi.org/10.1111/j.1365-2621.2003.tb09635.x