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*Full Length Article*

# Evaluation of Antioxidant, Antihypercholesterolemic and Cytotoxic Activity of Peptide Fractions from Melon (*Cucumis melo*) Seed

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## Abstract

Bioactive peptides are becoming more widely acknowledged as multifunctional substances that promote human health. Several reports revealed their therapeutic effects including antioxidant, anticancer, antihypertensive, cholesterol-lowering effects, etc. The present study aims to investigate the antioxidant, antihypercholesterolemic, and cytotoxic activities of peptides extracted from melon seed. The melon seeds were removed from fruits collected from a local market, dried and ground into powder. Protein hydrolysis was conducted by pepsin, thermolysin and trypsin digestion. Fractionation of the hydrolysate to obtain the peptide fractions (< 10 kDa, 10–30 kDa and > 30 kDa) was performed using ultrafiltration membranes. The fraction exhibiting the strongest antioxidant activity underwent further assays for anti-hypercholesterolemic and cytotoxic effects. It was observed that the fraction containing peptides of < 10 kDa molecular weight exhibited greatest antioxidant activity. Additionally, < 10 kDa fraction also showed antihypercholesterolemic and cytotoxic properties. Overall, this study suggests that peptides extracted from melon seed, particularly those with a molecular weight of < 10 kDa exhibit multifunctional activities, including antioxidant, antihypercholesterolemic, and cytotoxic properties, potentially conferring health benefits for humans.

**Keywords:** Melon seed; Peptide; Antioxidant; Antihypercholesterolemic; Cytotoxic

## Introduction

Numerous metabolic pathways can produce radical species or reactive oxygen species (ROS). Additionally, external factors, such as exposure to pollutants and unhealthy lifestyle play an important role in generating free radicals. Increased levels of these free radicals can trigger an imbalance of redox status in cells, causing oxidative stress. Excessive oxidative stress contributes to disruption of cell functions and the development of various diseases such as cardiovascular diseases, atherosclerosis, cancer, diabetes, neurological disorders (Mudgil *et al.* 2019; Chavda *et al.* 2022; Yan *et al.* 2023). Antioxidants can protect the human body against oxidative stress and may delay the progression of many diseases (Zhuang *et al.* 2013).

Various studies have shown that the compounds extracted from plant leaves and fruits exhibit antioxidant properties (Zhou *et al.* 2021; Asala *et al.* 2022; Feduraev *et al.* 2022). However, peptides are one class of bioactive molecules that have attracted a lot of attention recently. Bioactive peptides are known to exhibit diverse bioactivities such as antioxidative, antihypertensive, cholesterol-lowering, and antimicrobial (Mudgil *et al.* 2019). Peptides possessing multiple activities are favored over those with one single function as they have the capability to simultaneously regulate various physiological pathways (Aguilar-Toalá *et al.* 2017). Several studies have revealed that bioactive peptides with antioxidant properties can provide other bioactivities, such as anticancer (Chi *et al.* 2015) and cholesterol lowering effect (Siow *et al.* 2016).

Enzymatic hydrolysis is the most widely utilized technique in obtaining physiologically active peptides. This method has several advantages such as easy reaction control and mild condition. The utilization of distinct protease enzymes yields varying peptide compositions and sequences of amino acids due to disparities in the cleavage preferences of each protease type. Such discrepancies in the composition and sequence influence the bioactivities obtained (Nasri 2017). Various types of protease enzymes have been utilized to produce bioactive peptide including pepsin, trypsin, alcalase, flavourzyme and thermolysin *etc*.

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Melon (*Cucumis melo* L.) seed are byproducts of the fruit that have high protein content and potential as source of bioactive peptide. The protein content of melon seeds is 14.91–36.3% (Silva *et al.* 2020). According to Wen *et al.* (2020) protein resources found in byproducts or waste produced during plant processing have significant promise to produce bioactive peptides. Several studies have been exploring the biological activities of protein hydrolysate and peptide from seed of *Cucurbitaceae* plants. *In vitro* studies by Siddeeg *et al.* (2015) reported that protein hydrolysate of *C. melo* var. *tibish* have antioxidant properties. Wen *et al.* (2020) revealed that the peptide extracted from *Citrullus lanatus* (watermelon) seed possess antioxidant activity. Priyanto *et al.* (2015) revealed that peptides from *Momordica charantia* seed proteins can inhibit the activity of angiotensin-I converting enzyme (ACE). Furthermore, there are currently no studies available concerning the bioactivities of peptide fractions extracted from melon seed. Thus, the aim of this study was to evaluate the *in-vitro* antioxidant, antihypercholesterolemic and cytotoxic activity of peptide fractions from melon seed. Three different proteases were utilized to digest melon seed protein and ultrafiltration with a membrane molecular weight cut of (MWCO) 10 kDa and 30 kDa was performed to obtain the peptide fractions.

## Materials and Methods

### Materials

Melon (*Cucumis melo* L.) fruits were obtained from the local market. The seeds from fruits were removed and washed with distilled water. All chemicals used in this study were an analytical grade such as DPPH (Sigma Aldrich), ABTS (Sigma Aldrich), Ferrozine (Sigma Aldrich), Enzyme pepsin (Himedia), Enzyme trypsin (Himedia), Enzyme thermolysin (Sigma Aldrich), NaOH (Merck), HCl (Merck), NaCl (Merck), KCl (Merck), $Na_2HPO_4$ (Merck), $KH_2PO_4$ (Merck), MCF7 cell line (Elabscience).

### Melon seed preparation

The melon seeds were dried, ground into powder and defatted. Then protein from powder was isolated by following the method of Marques *et al.* (2015) with slight modifications. Briefly, the samples were suspended in an alkaline solution (1:10 w/v) pH 9.3. This mixture was stirred at room temperature for 2 h and then centrifuged at 4°C at a speed of 5500x for 15 min. The residue obtained was extracted again as before with half the volume of solvent from the first extraction. All the supernatants produced were combined and pH 4.4 was maintained by adding 1 *M* HCl solution. After centrifugation, the precipitates containing protein was isolated and the pH was adjusted to 7 by adding 1 *M* NaOH solution. The melon seed protein was then freeze dried (Buchi Lyovapor L-300) and stored at -20°C for further analysis.

### Fractionation of melon seed protein hydrolysate

The melon seed protein was digested by pepsin, thermolysin and trypsin prior to fractionation by following the hydrolysis procedure described by He *et al.* (2013) with slight modifications. Protein isolate (5% w/v) digested with proteases *i.e.*, trypsin (37°C and pH 8), pepsin (37°C, pH 2) and thermolysin (55°C, pH 8) for 4 h using enzyme-substrate ratio as 1:50. The enzyme activity was stopped by heating in a water bath at 88°C for 20 min. Subsequently, the mixture was centrifuged at 5500x for 30 min. The hydrolysate obtained was fractionated with sequential ultrafiltration using a 10 kDa and a 30 kDa ultrafiltration membrane (Millipore Amicon centrifugal filter unit (Merck). Initially, the hydrolysates passed through a filter membrane of 10 kDa and centrifugation. This step results in the permeate as fraction < 10 kDa and the retentate. The retentate was subsequently subjected to further ultrafiltration using a 30 kDa membrane, resulting in fraction 10–30 kDa (permeate) and fraction > 30 kDa (retentate). Thus, each hydrolysate yielded three fractions: < 10 kDa, 10–30 kDa and > 30 kDa.

### Antioxidant activity assay

**ABTS radical scavenging activity assay:** The ABTS (2,2'-azinobis-(3ethylbenzothiazoline)-6-sulfonic acid) radical scavenging properties of fractions were assessed following the procedure by Zhuang *et al.* (2013), with minor modifications. Briefly, 5 mL of 7 m*M* ABTS stock solution was mixed with 85 $\mu$L of 2.45 m*M* potassium persulfate and incubate for 14–16 h. The resulting ABTS radical solution was diluted with 0.2 *M* PBS pH 7.4 until its absorbance reached 0.7 at 734 nm. Subsequently, the fractions of sample (2 mg/mL) were combined with the diluted ABTS radical solution at a ratio of 1:4 and incubated for 6 min prior to measuring the absorbance. The mixture was then measured at a wavelength of 734 nm using microplate reader (BioTek Instrument Synergy H1MF microplate spectrophotometer) and PBS was taken as the blank. The percentage of radical inhibition was calculated using the provided equation:

$$\text{ABTS scavenging activity (\%)} = \frac{\text{absorbance of blank} - \text{absorbance of sample}}{\text{absorbance of blank}} \times 100$$

**DPPH radical scavenging activity assay:** DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay was conducted using Khaled *et al.* (2014) with slight modifications. Briefly, 375 $\mu$L of 90% ethanol, 500 $\mu$L of sample fractions (9 mg/mL) in 0.1 *M* phosphate buffer pH 7 and 125 $\mu$L of 0.4 m*M* DPPH in ethanol were mixed and incubated for an hour at 37°C. The absorbance was read at 517 nm. The formula given below was used to calculate the percentage of DPPH radical scavenging.

$$\text{DPPH scavenging activity (\%)} = \frac{\text{absorbance of blank} - \text{absorbance of sample}}{\text{absorbance of blank}} \times 100$$

**Metal ion chelating ability assay:** The ferrous ion chelate ability of the sample of peptide fractions was assessed following the procedure described by Yuan *et al.* (2019), with minor adjustments. Briefly, 100 $\mu$L of 2 m*M* $FeCl_2.4H_2O$ was combined with 1 mL of fractions (9 mg/mL) in sodium acetate buffer pH 5 and then incubated at 37°C for 30 min. 200 $\mu$L of 5 m*M* ferrozine was added to the mixture and subsequently incubated for 10 min at room temperature. The absorbance of the mixture was measured at 562 nm. The equation given below was used to determine chelating activity.

$$\text{Chelating activity (\%)} = \frac{\text{absorbance of blank} - \text{absorbance of sample}}{\text{absorbance of blank}} \times 100$$

**Antihypercholesterolemic activity assay**

**HMG-CoA reductase inhibition assay:** Inhibition of HMG-CoA reductase by samples was performed using the Sigma-Aldrich® HMG-CoA reductase assay kit (CS 1090-1KT) by following manufacturer's instructions. Briefly, 96-well plate was filled with each fraction sample, NADPH, substrate (HMG-CoA) and buffer. HMG-CoA reductase was added to each well to start the reactions, and the absorbance was measured at 340 nm. The decrease in absorbance over a period of 10 min was measured which being directly correlated with HMG-CoA reductase activity. The HMG-CoA reductase activity was denoted as $\mu$moL of oxidized NADPH/min/mgP. The equation below was used to determined percentage of HMG CoA reductase inhibition by the samples.

$$\text{Inhibition (\%)} = \frac{\text{UMG CoA reductase control activity HMG CoA reductase in the presence of sample}}{\text{HMG CoA reductase control activity}} \times 100$$

**Bile acid binding activity assay:** The bile-acid binding capacity was conducted following the method by Yoshie-Stark and Wäsche (2004) with minor modification. Briefly, 2 m*M* sodium deoxycholate as bile acid solution was combined with fractions (10 mg/mL) in 0.1 m*M* phosphate buffer pH 7. The mixture was incubated for 2 h at 37°C and then centrifugation was performed at 12000 rpm for 10 min. The bile acid content in the fraction sample was analyzed according to the instructions provided in the Bile Acid kit (MAK309 from Sigma Aldrich). All samples were analyzed in duplicate using fluorescence spectrophotometry at an excitation wavelength of 530 nm and an emission wavelength of 585 nm. The following equation was used to calculate the bile acid binding activity:

$$\text{Bile acid binding (\%)} = \frac{\text{absorbance of blank} - \text{absorbance of sample}}{\text{absorbance of blank}} \times 100$$

Cytotoxic activity assay: The cytotoxic activity of peptide fractions was assessed following the procedure described by Arsianti *et al.* (2022), with slight adjustments. MCF7 cells line were loaded into 96-plate wells at a density of $1x10^4$ cells/well and incubated in a 5% $CO_2$ incubator at 37°C until grown. Then, the hydrolysate fractions of various concentrations (2, 4, 6, 8 and 10 mg/mL) with PBS solvent control were added to wells. Cells were then incubated for 24 h at 37ºC. At the end of incubation, the sample was discarded and then to each well, 100 $\mu$L of 0.5 mg/mL MTT was added. Cells were incubated again in a 5% $CO_2$ incubator for 4 h at 37ºC. The MTT reaction was stopped with DMSO. Absorbance was read with an ELISA reader at a wavelength of 590 nm. The percentage of cell viability was calculated using the following formula:

$$\text{Cell viability (\%)} = \frac{\text{absorbance of control} - \text{absorbance of medium}}{\text{absorbance of sample} - \text{absorbance of medium}} \times 100$$

**Determination of protein content and amino acids composition**

The protein content of samples was determined using the Bradford method and the amino acids analysis was conducted using the UPLC system according to System Guide of Waters Acquity UPLC H Class and H Class Bio amino Acid Analysis. 1 µL of each sample was injected into column (AccQ.Tag Ultra C18 1.7 $\mu$m (2.1 x 100 mm), with PDA detection at 260 nm wavelength and temperature 49°C. The flow rate of mobile phase was 0.5 mL/min with following compositions: AccQ.Tag Ultra amino acid analysis eluent A; AccQ.Tag Ultra amino acid analysis 10% (in water), Aquabidest grade HPLC, and AccQ.Tag Ultra amino acid analysis eluent B (as mobile phase A, mobile phase B, mobile phase C and mobile phase D, respectively).

**Statistical analysis**

The data from each assay were examined for standard deviation, and the results shown represent the mean ± standard deviation. A one-way analysis of variance (ANOVA) was conducted using IBM SPSS Statistics version 23 to assess significant differences among the means, with Tukey's test applied at a significance level of 0.05.

## Results

**Antioxidant activity of melon seed peptide fractions**

Fractions were tested for antioxidant activity using the ABTS, DPPH and $Fe^{2+}$ metal chelation. The results showed that the fractions of melon seed protein hydrolysate have antioxidant activity. In general, < 10 kDa fraction produced greater scavenging radical and chelating activities than the 10–30 kDa fraction and the > 30 kDa fraction as presented in Table 1. The fraction that showed the greatest antioxidant activity was then tested for antihypercholesterolemic and cytotoxic properties.

**Anti-hypercholesterolemic activity of < 10 kDa fractions**

The < 10 kDa peptide fraction from melon seeds (2 mg/mL) can inhibit HMG CoA reductase in the range 51.42% ± 9.29% to 72.04% ± 1.37%. Bile acid binding ability of the fraction (10 mg/mL) ranges from 21.65% ± 1.99% to 32.98% ± 1.99% (Table 2). The results showed that the thermolysin fraction had the best anti-

**Table 1:** Antioxidant activity of peptide fractions from melon seeds

| Enzyme | Fraction | ABTS radical scavenging (%) | DPPH radical scavenging (%) | Metal chelating activity (%) |
|---|---|---|---|---|
| Pepsin | < 10 kDa | $25.75 \pm 1.69^{a}$ | $16.04 \pm 0.48^{a}$ | $13.06 \pm 0.75^{a}$ |
| | 10-30 kDa | $10.73 \pm 2.66^{b}$ | $5.85 \pm 1.40^{bde}$ | $9.91 \pm 0.84^{b}$ |
| | > 30 kDa | $18.99 \pm 0.38^{c}$ | $5.72 \pm 0.73^{bde}$ | $24.07 \pm 0.52^{c}$ |
| Thermolysin | < 10 kDa | $37.45 \pm 2.54^{d}$ | $20.95 \pm 1.39^{c}$ | $53.09 \pm 0.49^{d}$ |
| | 10-30 kDa | $9.65 \pm 2.01^{b}$ | $4.42 \pm 0.73^{bd}$ | $13.09 \pm 0.48^{a}$ |
| | > 30 kDa | $22.78 \pm 0.19^{ac}$ | $6.39 \pm 1.26^{be}$ | $38.78 \pm 0.68^{e}$ |
| Trypsin | < 10 kDa | $29.75 \pm 1.75^{e}$ | $18.67 \pm 0.51^{f}$ | $67.20 \pm 0.57^{f}$ |
| | 10-30 kDa | $9.13 \pm 2.69^{b}$ | $7.33 \pm 0.52^{be}$ | $25.92 \pm 0.77^{c}$ |
| | > 30 kDa | $21.74 \pm 0.45^{c}$ | $5.32 \pm 0.75^{bde}$ | $41.29 \pm 2.90^{g}$ |

All values are mean ± standard deviation. Mean in the same column with different letters are significantly different ($P < 0.05$)

**Table 2:** Anti-hypercholesterolemic activity of the < 10 kDa peptide fraction from melon seeds

| Fraction < 10kDa | HMG CoA reductase inhibition (%) | Bile acid binding (%) |
|---|---|---|
| Pepsin | $51.42 \pm 9.29^{a}$ | $24.60 \pm 0.72^{a}$ |
| Thermolysin | $72.04 \pm 1.37^{b}$ | $32.98 \pm 1.99^{ab}$ |
| Trypsin | $57.94 \pm 6.68^{ab}$ | $21.65 \pm 1.99^{ac}$ |

All values are mean ± standard deviation. Mean in the same column with different letters are significantly different ($P < 0.05$)

hypercholesterolemic properties.

### Cytotoxic activity of < 10 kDa fractions

The < 10 kDa fractions of melon seed could inhibit MCF7 cells growth. The viability of cancer cells treated with the fraction depends on its concentration. Higher concentrations of the fraction result in decreased viability of the cancer cells (Fig. 1). At a concentration of 10 mg/mL, the percent viability of MCF7 cells is in the range of 46.30% ± 0.12% to 56.6 ± 0.33%. Morphological changes were observed in MCF7 cells treated with fraction samples compared to the control, as shown in Fig. 2.

### Protein content and amino acids composititon of < 10 kDa fractions

The amino acid profiles of the < 10 kDa fractions can be observed in Table 3, where the samples exhibit corresponding profiles. Arginine, glutamic acid, aspartic acid, phenylalanine, and leucine are the most abundant amino acids. The protein content of each fraction based on Bradford assay is 227.14 ± 8.16 mg/mL, 210.95 ± 17.81 mg/mL and 499.04 ± 20.54 mg/mL for pepsin, thermolysin and trypsin, respectively.

## Discussion

Many reports have revealed the bioactivities of peptides from various sources of protein. In this study, the peptide fractions from melon seed protein showed multifunctional bioactivities. Peptide fractions with a low molecular weight (< 10 kDa) generally have better antioxidant activity than peptides with a larger molecular weight. According to Xia *et al.* (2012), this is because smaller peptides interact more easily with radical species. A study by Siow and Gan (2016) also showed that the < 10 kDa fraction of protein hydrolysate

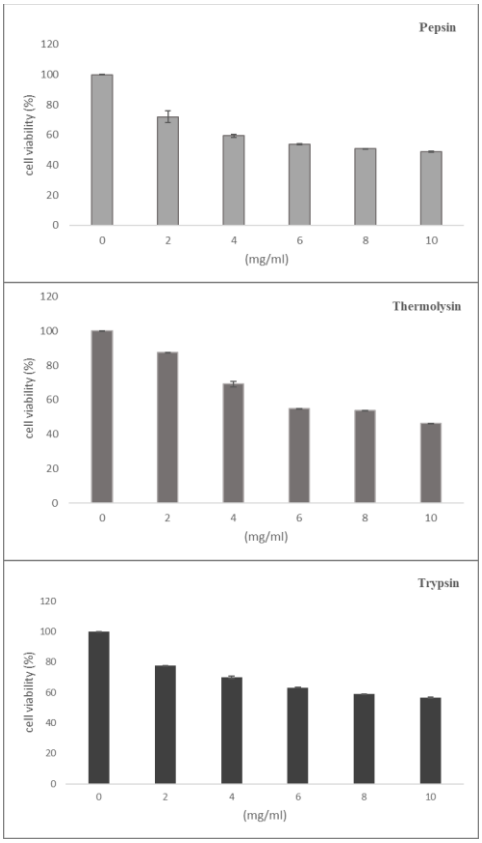


**Fig. 1:** Cytotoxic activity of < 10 kDa peptide fractions from melon seed

of *Parkia speciosa* seeds had better antioxidant activity than the 10–30 kDa, 30–50 kDa and > 50 kDa fractions.

The amino acid composition of < 10 kDa fractions (Table 3), contain antioxidant amino acids including tyrosine, histidine, phenylalanine, and threonine. Histidine has radical scavenging activity due to the imidazole group

which can donate protons. The resulting amino acids such as tyrosine and tryptophan can eradicate radicals because they each have hydroxyphenyl and indole groups which can donate hydrogen atoms and turn into phenoxy radicals or indole radicals. This second radical can be stabilized because there is a conjugated electron system in its structure (Wu *et al.* 2015). This study also revealed that fraction having < 10 kDa peptides isolated using thermolysin had higher scavenging radical ABTS and DPPH than those obtained using pepsin and trypsin. Differences in protease specificity towards protein substrates affect the composition and sequence of generated peptides, thereby influencing differences in bioactivity (Nasri 2017).

Peptides are one of the best metal chelators because they have carboxyl, amino and side chain groups that can be involved in binding divalent cations. The electron donating group of peptides can act as a Lewis base that interacts with metal ions as electron acceptors through coordination bond (Caetano-Silva *et al.* 2021). According to Li *et al.* (2017), peptides with a smaller size have a greater number of terminal carboxyl and amino terminal groups than large peptides, which may result in a higher metal chelating ability. Large peptides may also exhibit chelating activity resulting in a larger number of dentate areas. The best chelator activity in this study is shown by fraction from trypsin digestion. This can be due to trypsin produce peptides with positively charged amino acids, namely arginine and lysine, at the C terminus (Olsen *et al.* 2004). The side chains of arginine and lysine contain nitrogen which is the main electron donor in the formation of peptide complexes with metals. The nitrogen atom in lysine, arginine or histidine is the main part that binds $Fe^{2+}$ ions (Tian *et al.* 2023). Study by Torres-Fuentes *et al.* (2012) revealed that the peptide fraction of chickpea protein hydrolysate which was rich in arginine and lysine produced greater $Fe^{2+}$ ion chelation activity than the other fractions.

In this currently study, the < 10 kDa fraction which showed the best antioxidant activity was tested for anti-hypercholesterolemic and cytotoxic activity. The peptide fraction from protein hydrolysis by thermolysin showed greater bioactivities which could be caused by differences in the composition, size and sequence of bioactive peptides produced by each fraction. In this study, the *in vitro* antihypercholesterolemic activity was assessed using HMG-CoA reductase inhibition and bile acid binding, which substances known to influence blood cholesterol levels in the human body. To function as a competitive inhibitor of HMGR, the peptide must imitate the conformation or structure of HMG, which may depend on the conformation and structure of the side chains of the amino acids of the peptide (Lammi *et al.* 2019). Moreover, polar, and non-polar groups of amino acids in peptide may interact with bile acid *via* hydrophilic and hydrophobic interactions. Bile acid structure consists of hydrophobic steroid core, hydrophilic hydroxyl, and carboxyl groups. These groups facilitate the binding to other molecules including peptides (Siow *et al.* 2016).

**Table 3:** Amino Acids composition of the < 10 kDa peptide fraction from melon seeds (mg/kg sample)

| Amino Acids | Pepsin | Thermolysin | Trypsin |
|---|---|---|---|
| Serine | 1674.54[a] | 2524.265[b] | 2459.955[c] |
| Glutamic acid | 4540.97[a] | 7067.88[b] | 7107.595[b] |
| Phenylalanine | 2509.43[a] | 3954.06[b] | 3338.165[c] |
| Isoleucine | 1093.02[a] | 1746.265[b] | 1470.59[c] |
| Valine | 1306.72[a] | 2049.07[b] | 1699.345[c] |
| Alanine | 1248.24[a] | 1940.5[b] | 1658.385[c] |
| Arginine | 4885.31[a] | 7796.35[b] | 8680.95[c] |
| Glycine | 1323.79[a] | 2001.965[b] | 1916.74[c] |
| Lysine | 645.80[a] | 1207.195[b] | 1004.12[c] |
| Aspartic acid | 2438.82[a] | 3710.47[b] | 3165.77[c] |
| Leucine | 2092.71[a] | 3248.035[b] | 2763.37[c] |
| Tyrosine | 1209.69[a] | 2113.395[b] | 1636.86[c] |
| Proline | 1064.22[a] | 1561.085[b] | 1450.83[c] |
| Threonine | 1246.35[a] | 1977.005[b] | 1809.415[c] |
| Histidine | 894.52[a] | 1483.62[b] | 1379.965[c] |

All values are mean ± standard deviation. Mean in the same row with different letters correspond to significant differences ($P < 0.05$)

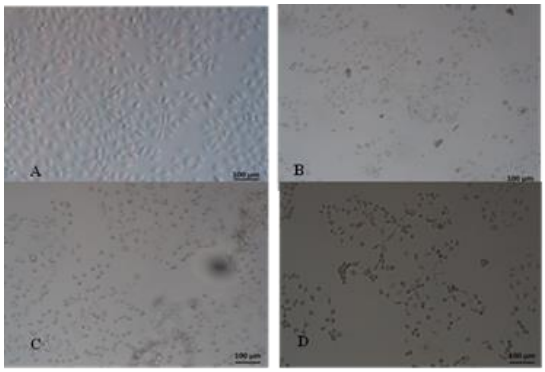


**Fig. 2:** Morphology of MCF7 cells treated with < 10 kDa peptide fraction from melon seeds. **A**: control, **B**: pepsin, **C**: trypsin, **D**: thermolysin

The cytotoxic properties of peptides against cancer cells have the potential to serve as anticancer activity. The amino acids in peptides can provide anticancer effects by several mechanisms. Chiangjong *et al.* (2020) revealed that positively charged amino acids, such as arginine, lysine, and histidine, can disrupt cancer cell membranes. Phenylalanine and hydrophobic amino acids can increase affinity for cell membranes and help peptide penetration into the hydrophobic part of cancer cell membranes in the form of lipid molecules. Furthermore, bioactive peptides may induce apoptosis of cancer cells via caspase-3 activation and down-regulation of Bcl-2 (Gupta and Bhagyawant 2021). This study demonstrates that treatment with < 10 kDa peptide fractions from melon seeds can induce morphological changes in MCF7 cells (Fig. 2).

Several previous studies have also documented the multifunctional bioactivities of peptides. Studies by Siow *et al.* (2016) showed that bioactive peptides from cumin seeds have antioxidant activity, inhibit the amylase enzyme, and have the potential to reduce cholesterol and binding bile acids. A report

by Homayouni-Tabrizi *et al.* (2017) showed that camel milk peptides have cytotoxic and antioxidant activity.

## Conclusion

The current research showed that the < 10 kDa peptide fraction of protein hydrolysate from melon seed has greater antioxidant activity compared to 10–30 kDa and > 30 kDa. The < 10 kDa fractions demonstrated *in vitro* antihypercholesterolemic and cytotoxic activity. Further investigations are required to find the sequence of amino acids in the melon seed peptide fraction. Morover, *in vivo* and *in silico* studies are necessary to enhance the findings obtained in the present study.

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## Author Contributions

All authors have contributed to the development of this work and have permitted it to be published.

## Conflicts of Interest

All authors declare no conflict of interest.

## Data Availability

The data of this study will be provided to access upon a reasonable request to the corresponding author.

## Ethics Approval

Outside the scope of this paper.

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