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# Anti-proliferative potential of seed derived proteins from *Vitis vinifera* and *Mangifera indica*

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**ABSTRACT**

The present study focused on the extraction, purification, and characterization of proteins from seeds of *Vitis vinifera* and *Mangifera indica*, along with evaluation of their anti-proliferative potential. Protein estimation using the Lowry method revealed higher protein content in grape seed extract (4.98 ± 0.14 mg/mL) compared to mango seed extract (4.20 ± 0.12 mg/mL). Spectrophotometric analysis at 280 nm showed higher absorbance for grape seed protein (1.32 ± 0.05) than mango seed protein (1.08 ± 0.04), confirming greater protein abundance. SDS-PAGE profiling indicated multiple protein bands in the range of 30–97 kDa for grape seeds and 35–80 kDa for mango seeds, with grape seed proteins exhibiting greater molecular diversity. The anti-proliferative activity of the purified proteins was assessed using the MTT assay against HeLa and MCF-7 cancer cell lines. A dose-dependent decrease in cell viability was observed, with HeLa cells showing higher sensitivity ($IC_{50}$ ≈ 120 µg/mL) compared to MCF-7 cells ($IC_{50}$ ≈ 135 µg/mL). At 200 µg/mL, cell viability decreased to 29.5% in HeLa and 35.0% in MCF-7 cells. Morphological assessment uses light microscopy and acridine orange/ethidium bromide staining revealed characteristic apoptotic features, including cell shrinkage, membrane blebbing, chromatin condensation, and nuclear fragmentation, confirming apoptosis as the primary mechanism of cell death. Overall, the results indicate that grape seed proteins possess higher protein content, greater structural complexity, and stronger anti-proliferative activity compared to mango seed proteins. These findings highlight the potential of seed-derived proteins as promising natural candidates for anticancer applications and support further investigation into their molecular mechanisms.

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## INTRODUCTION

Plant-derived proteins have attracted increasing attention due to their diverse biological activities and potential applications in pharmaceutical and nutraceutical fields. Seeds, in particular, serve as rich reservoirs of storage proteins and bioactive molecules that play crucial roles in plant growth, development, and defense. These proteins have been widely explored for their structural, functional, and therapeutic significance (Shewry *et al.*, 1995). Seeds of *Vitis vinifera* and *Mangifera indica* are abundant agro-industrial by-products that remain underutilized despite their biochemical richness. Grape seeds are known to contain a variety of bioactive compounds, including proteins and peptides, in addition to well-studied polyphenols, contributing to their functional properties (Shi *et al.*, 2003). Mango seed kernels also contain proteins along with carbohydrates and lipids; although their protein composition and extractability are comparatively lower (Soong and Barlow, 2004).

The extraction and purification of plant proteins are essential steps for their characterization and evaluation of biological activities. Techniques such as ammonium sulfate precipitation, dialysis, and chromatographic separation are commonly employed to isolate proteins with improved purity and functional properties (Scopes, 1994). Analytical methods including spectrophotometry and SDS-PAGE further facilitate the identification and characterization of protein fractions based on their concentration and molecular weight distribution. In recent years, plant-derived proteins have been reported to exhibit significant anti-proliferative activity against various cancer cell lines. These proteins, including lectins and bioactive peptides, can interact with cellular components, leading to inhibition of cell growth and induction of apoptosis (Lam and Ng, 2011). Apoptosis, a programmed cell death mechanism, is characterized by distinct morphological and biochemical changes such as cell shrinkage, membrane blebbing, chromatin condensation, and nuclear fragmentation.

Despite the growing interest in plant proteins, limited studies have focused on the systematic extraction, purification, and biological evaluation of proteins from grape and mango seeds, particularly in relation to their anti-proliferative potential. Therefore, the present study aims to isolate and characterize proteins from these seed sources and to assess their cytotoxic effects using cancer cell lines, along with morphological analysis to elucidate the mode of cell death. The present study focused on the extraction, purification, and characterization of proteins from seeds of *Vitis vinifera* and *Mangifera indica*, along with evaluation of their anti-proliferative potential.

## MATERIALS AND METHODS

### Collection and preparation of plant material

Seeds of *Vitis vinifera* and *Mangifera indica* were collected from local sources. The seeds were washed thoroughly with distilled water to remove adhering pulp and impurities. The cleaned seeds were shade-dried at room temperature for 5–7 days. Dried seeds were then ground into fine powder using a laboratory grinder and stored in airtight containers at 4°C for further analysis (Harborne, 1998).

### Extraction of crude protein

Protein extraction was carried out using phosphate buffer. Approximately 5 g of seed powder was homogenized in 50 mL of 0.05 M phosphate buffer (pH 7.0) containing 0.5 M NaCl and 1 mM EDTA to enhance protein solubility and prevent proteolytic degradation. The homogenate was stirred continuously for 2–4 hours at 4°C and then centrifuged at 10,000 rpm for 20 minutes at 4°C. The supernatant obtained was collected and used as crude protein extract. Proteins were precipitated using 70–80% ammonium sulfate saturation, followed by overnight incubation at 4°C. The precipitate was collected by centrifugation and dialyzed against phosphate buffer for 24 h (Scopes, 1994).

### Protein precipitation by ammonium sulfate fractionation

The crude extract was subjected to ammonium sulfate precipitation to concentrate proteins. Solid ammonium sulfate was slowly added to the extract with continuous stirring at 4°C to achieve 70–80% saturation. The mixture was incubated overnight at 4°C and centrifuged

at 10,000 rpm for 20 minutes. The resulting precipitate was collected and dissolved in a minimal volume of phosphate buffer (pH 7.0) (Hughes, 1935).

**Dialysis**

The protein solution obtained after precipitation was subjected to dialysis to remove excess salts. The sample was placed in a dialysis membrane (molecular weight cut-off ~10 kDa) and dialyzed against phosphate buffer (pH 7.0) for 24 hours at 4°C with periodic buffer changes (Burgess, 2009).

**Purification of protein**

*Ion exchange chromatography*

Partial purification of proteins was carried out using DEAE-cellulose column chromatography. The column was equilibrated with phosphate buffer (pH 7.0), and the dialyzed protein sample was loaded onto the column. Proteins were eluted using a linear gradient of NaCl (0–1 M). Fractions were collected and monitored by measuring absorbance at 280 nm (Peterson and Sober, 1956).

*Gel filtration chromatography*

Further purification was achieved using gel filtration chromatography with Sephadex G-75. The column was equilibrated with phosphate buffer, and the protein sample was applied. Fractions were collected and analyzed for protein content based on absorbance at 280 nm (Andrews, 1964).

**Protein characterization and analysis**

*Estimation of protein content*

Protein concentration was determined using the Lowry method (Lowry *et al.*, 1951). Aliquots of protein samples were mixed with alkaline copper reagent and incubated for 10 minutes. Folin–Ciocalteu reagent was then added, and the mixture was incubated in the dark for 30 minutes. Absorbance was measured at 660 nm using a spectrophotometer. Bovine Serum Albumin (BSA) was used as the standard for calibration.

*Spectrometric analysis*

The presence of protein in the samples was confirmed using UV–Visible spectrophotometry. Absorbance of the protein solution was recorded at 280 nm, which corresponds to the presence of aromatic amino acids such as tyrosine and tryptophan (Layne, 1957).

*SDS-PAGE analysis*

Protein profiling and molecular weight determination were performed using Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) following the method of Laemmli (1970). A resolving gel (10–12%) and stacking gel (5%) were prepared. Protein samples were mixed with loading buffer containing SDS and β-mercaptoethanol and heated at 95°C for 5 minutes to denature the proteins. The samples and molecular weight marker were loaded into the wells, and electrophoresis was carried out at 80 V for stacking and 120 V for resolving. After electrophoresis, the gel was stained with Coomassie Brilliant Blue and destained until clear protein bands were visible. The molecular weights of the proteins were estimated by comparing their mobility with standard protein markers.

**Biological activity assays**

*Anti-proliferative activity (MTT Assay)*

The anti-proliferative activity of the protein extract was assessed using the MTT assay (Mosmann, 1983). Cancer cells (HeLa and MCF-7) were seeded in 96-well plates and incubated at 37°C in a humidified atmosphere with 5% $CO_2$ for 24 h. Cells were treated with varying concentrations of protein extract and incubated for 24–48 h. Subsequently, MTT reagent was added and incubated for 4 h. The resulting formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm. Cell viability was expressed as a percentage relative to untreated control.

*Morphological assessment of apoptotic cells*

Morphological changes in treated cells were evaluated using both light and fluorescence microscopy. For light microscopy, cells were fixed with methanol and stained with Giemsa or crystal violet, followed by observation for apoptotic features such as cell shrinkage and membrane blebbing. For fluorescence microscopy, treated cells were stained with acridine orange/ethidium bromide (AO/EtBr) and observed

under a fluorescence microscope. Viable cells exhibited green fluorescence, whereas apoptotic cells showed orange to red fluorescence with condensed or fragmented nuclei (Ribble *et al.*, 2005).

**Statistical analysis**

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation. Statistical analysis was performed using SPSS (Zar, 2010).

## RESULTS

**Protein extraction**

Protein extraction from seeds of *Vitis vinifera* and *Mangifera indica* yielded appreciable amounts of soluble proteins. The quantitative estimation of protein revealed that the grape seed extract exhibited a higher protein concentration (3.85 ± 0.12 mg/mL) compared to the mango seed extract (3.12 ± 0.10 mg/mL). The difference in protein content between the two samples may be attributed to variations in seed composition, extraction efficiency, and inherent biochemical properties of the plant materials. The relatively higher protein yield observed in grape seeds suggests a greater abundance of soluble proteins, which may contribute to enhanced biological activities (Table 1).

**Purification and separation of plant protein**

The purification of seed proteins from *Vitis vinifera* and *Mangifera indica* was achieved through sequential steps including ammonium sulfate precipitation, dialysis, and column chromatography. The effectiveness of each purification step was evaluated based on total protein content and specific activity, as presented in Table 2. The purification process resulted in a progressive increase in specific activity, indicating effective enrichment of the target protein. The crude extract exhibited a total protein content of 100 mg with a low specific activity of 1.2 U/mg, reflecting the presence of a large proportion of non-target proteins and impurities.

**Table 1.** Total protein content of seed extracts

| Sample | Protein concentration (mg/mL) |
|---|---|
| *Vitis vinifera* seed protein | 3.85 ± 0.12 |
| *Mangifera indica* seed protein | 3.12 ± 0.10 |

**Table 2** Purification profile of seed proteins

| Step | Total protein (mg) | Specific activity (U/mg) |
|---|---|---|
| Crude extract | 100 | 1.2 |
| Ammonium sulfate fraction | 65 | 2.8 |
| Dialyzed sample | 52 | 3.5 |
| Purified fraction | 30 | 5.6 |

**Table 3.** Protein estimation and spectrometric analysis

| Sample | Protein concentration (mg/mL) | Absorbance at 280 nm |
|---|---|---|
| *Vitis vinifera* seed protein | 4.98 ± 0.14 | 1.32 ± 0.05 |
| *Mangifera indica* seed protein | 4.20 ± 0.12 | 1.08 ± 0.04 |

Following ammonium sulfate precipitation, the total protein content decreased to 65 mg, while the specific activity increased to 2.8 U/mg. This step selectively precipitated proteins based on solubility, thereby removing a portion of unwanted proteins and enhancing enzyme purity.

Subsequent dialysis further reduced the total protein to 52 mg, with an increase in specific activity to 3.5 U/mg. The removal of salts and low-molecular-weight contaminants during dialysis contributed to improved protein purity.

The final purification step using column chromatography resulted in a significant increase in specific activity to 5.6 U/mg, despite a reduction in total protein to 30 mg. This indicates a high degree of purification, with most non-specific proteins eliminated and the target protein enriched (Table 2).

**Protein estimation**

The protein concentration determined using the Lowry method revealed that grape seed extract exhibited a higher protein content (4.98 ± 0.14 mg/mL) compared to mango seed extract (4.20 ± 0.12 mg/mL). The results indicate efficient extraction and purification of proteins, with reproducible values reflected by low standard deviations.

**Spectrometric analysis**

Spectrometric analysis further confirmed the presence of proteins in both samples, as evidenced by significant absorbance at 280 nm, corresponding to aromatic amino acids such as tyrosine and tryptophan. Grape seed protein showed higher absorbance (1.32 ± 0.05) than mango seed protein (1.08 ± 0.04), which correlates with its higher protein concentration (Table 3).

**SDS-PAGE analysis**

SDS-PAGE analysis revealed distinct protein banding patterns, indicating the presence of multiple protein fractions. The grape seed protein displayed four prominent bands within the molecular weight range of 30–97 kDa, with major bands observed at approximately 25, 45, and 60 kDa. In contrast, mango seed protein exhibited three bands ranging from 35–80 kDa, with prominent bands around 30 and 50 kDa. The presence of discrete bands confirms the partial purification of proteins and indicates heterogeneity in protein composition. The relatively fewer bands observed in mango seed extract suggest comparatively lower protein complexity or effective removal of minor proteins during purification (Fig. 1).

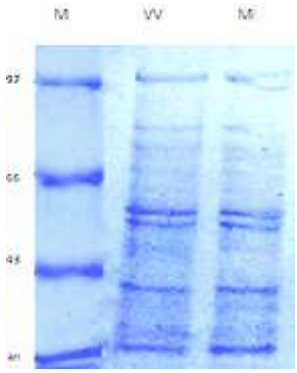


**Fig. 1.** SDS-PAGE analysis of purified proteins

**Anti-proliferative activity**

*Vitis vinifera seed protein*

The anti-proliferative activity of *Vitis vinifera* seed protein was evaluated against HeLa and MCF-7 cell lines using the MTT assay. The results demonstrated a clear dose-dependent reduction in cell viability in both cell lines with increasing concentrations of the protein extract (7.8–500 µg/mL). In HeLa cells, the percentage of viable cells decreased from 90.5 ± 2.1% at 7.8 µg/mL to 9.5 ± 0.8% at 500 µg/mL, with a corresponding increase in cell inhibition from 9.5 ± 2.1% to 90.5 ± 0.8%. The $IC_{50}$ value was estimated to be approximately 46.85 µg/mL, indicating a relatively higher sensitivity of HeLa cells to the extract.

Similarly, in MCF-7 cells, cell viability decreased from 92.8 ± 2.2% at 7.8 µg/mL to 12.4 ± 1.0% at 500 µg/mL, while cell inhibition increased from 7.2 ± 2.2% to 87.6 ± 1.0%. The $IC_{50}$ value was determined to be approximately 59.3 µg/mL, suggesting moderate cytotoxic activity compared to HeLa cells. The positive control exhibited 100% cell viability, whereas the negative control (standard drug) showed maximum inhibition with viability reduced to 5.3 ± 0.5% in HeLa and 6.2 ± 0.6% in MCF-7 cells, confirming the validity of the assay (Fig. 2).

*Mangifera indica seed protein*

The anti-proliferative activity of *Mangifera indica* seed protein was assessed against HeLa and MCF-7 cell lines using the MTT assay. The results revealed a concentration-dependent decrease in cell viability in both cell lines, indicating the cytotoxic potential of the extract. In HeLa cells, cell viability decreased progressively from 92.6 ± 2.2% at 7.8 µg/mL to 12.6 ± 1.0% at 500 µg/mL, accompanied by an increase in cell inhibition from 7.4 ± 2.2% to 87.4 ± 1.0%. The $IC_{50}$ value was estimated to be approximately 50–55 µg/mL, suggesting moderate cytotoxic efficacy.

Similarly, in MCF-7 cells, viability declined from 94.8 ± 2.3% at 7.8 µg/mL to 15.5 ± 1.1% at 500 µg/mL, while cell inhibition increased from 5.2 ± 2.3% to 84.5 ± 1.1%. The $IC_{50}$ value was estimated to be approximately 60–65 µg/mL, indicating comparatively

lower sensitivity of MCF-7 cells to the extract than HeLa cells. The positive control maintained 100% cell viability, whereas the negative control (standard drug) exhibited maximum cytotoxicity, reducing viability to 5.3 ± 0.5% in HeLa and 6.2 ± 0.6% in MCF-7 cells, thereby validating the experimental conditions (Fig. 2).

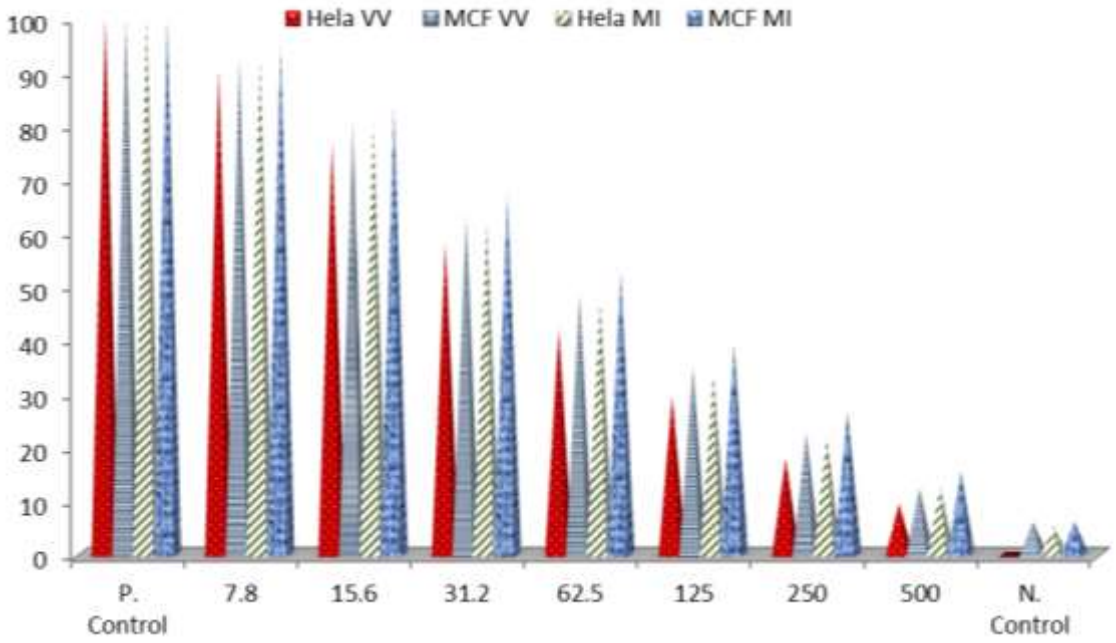


**Fig. 2.** Analysis of anti-proliferative of *Vitis vinifera* and *Mangifera indica* seed protein on HeLa and MCF-7 cells using MTT assay

**Table 4.** $IC_{50}$ values of cytotoxic activity of *Vitis vinifera* and *Mangifera indica* seed proteins against HeLa and MCF-7 cell lines

| Cell lines | Cytotoxicity activity | |
|---|---|---|
| | *Vitis vinifera* $IC_{50}$ (µg/mL) | *Mangifera indica* $IC_{50}$ (µg/mL) |
| HeLa | 46.85±6.4 | 55±7. 6 |
| MCF-7 | 59.3±9.2 | 65±10.2 |

In both cell lines, a clear dose-dependent decrease in viability and corresponding increase in inhibition were observed for both extracts. However, *Vitis vinifera* consistently exhibited lower $IC_{50}$ values, indicating higher potency. Additionally, HeLa cells were more responsive than MCF-7 cells to both treatments, suggesting cell line-specific sensitivity. Overall, while both seed proteins possess significant anti-proliferative properties, *Vitis vinifera* demonstrates comparatively higher cytotoxic efficacy than *Mangifera indica*, making it a more promising candidate for further anticancer investigations (Table 4).

**Morphological assessment**

Morphological analysis of control and protein-treated cells was carried out using light microscopy and fluorescence microscopy (AO/EtBr staining). The observations revealed clear differences between untreated and treated cells, indicating the induction of apoptosis by the protein extract in a concentration-dependent manner. The morphological alterations observed in treated cells were assessed to evaluate the cytotoxic and apoptotic effects of the test samples. In the control group, cells exhibited normal morphology characterized by a typical elongated shape, strong adherence to the culture surface, intact membrane integrity, and well-defined nuclei. The fluorescence staining showed uniform green coloration, indicating viable cells with intact membranes. Upon treatment at the $IC_{50}$ concentration, noticeable morphological changes were observed. Cells became rounded with a moderate loss of adherence, suggesting the initiation of

cytotoxic effects. Membrane blebbing was evident, indicating early stages of apoptosis. Nuclear morphology showed condensation, a hallmark of apoptotic progression. Fluorescence analysis revealed a shift from green to yellow/orange, reflecting compromised membrane integrity and early apoptotic cell population. At a higher concentration (200 µg/mL), the morphological changes were more pronounced. Cells exhibited severe shrinkage and were largely detached from the substrate, indicating advanced cytotoxicity. Extensive membrane blebbing was observed, along with nuclear fragmentation, confirming late-stage apoptosis. The fluorescence color transitioned predominantly to orange/red, signifying loss of membrane integrity and increased uptake of ethidium bromide, which is characteristic of late apoptotic or necrotic cells (Table 5).

**Table 5.** Morphological changes in cells at different treatment concentrations assessed by AO/EtBr staining

| Parameter | Control | $IC_{50}$ µg/mL | 200 µg/mL |
|---|---|---|---|
| Cell shape | Normal | Rounded | Severe shrinkage |
| Cell adherence | Strong | Moderately reduced | Detached |
| Membrane integrity | Intact | Blebbing evident | Extensive blebbing |
| Nuclear morphology | Intact | Condensed | Fragmented |
| Fluorescence color | Green | Yellow/orange | Orange/red |

**Table 6.** Effect of *Vitis vinifera* and *Mangifera indica* seed proteins on nuclear morphological changes in HeLa and MCF-7 cells assessed by AO/EtBr staining

| Treatment groups | HeLa | | MCF-7 | |
|---|---|---|---|---|
| | $IC_{50}$ | 200 µg/mL | $IC_{50}$ | 200 µg/mL |
| No. of apoptotic cells / 100 cells | | | | |
| *V. vinifera* protein extract | 24.2 ± 2.1[a] | 67.4 ± 3.2[b] | 46.5 ± 2.8[a] | 77.3 ± 3.5[b] |
| *M. indica* protein extract | 38.3 ± 2.5[a] | 72.5 ± 3.6[b] | 35.2 ± 2.4[a] | 69.5 ± 3.1[b] |
| Apoptotic ratio | | | | |
| *V. vinifera* protein extract | 0.31 ± 0.02[a] | 2.06 ± 0.15[b] | 0.86 ± 0.06[a] | 3.40 ± 0.20[b] |
| *M. indica* protein extract | 0.62 ± 0.04[a] | 2.63 ± 0.18[b] | 0.54 ± 0.05[a] | 2.27 ± 0.16[b] |

**Apoptotic activity**

The effect of *Vitis vinifera* and *Mangifera indica* seed proteins on nuclear morphological alterations in HeLa and MCF-7 cells was evaluated using AO/EtBr dual staining. The results demonstrated a marked increase in apoptotic cell population in both cell lines following treatment, confirming the pro-apoptotic potential of the protein extracts. In HeLa cells, treatment with *Vitis vinifera* protein extract resulted in 24.2 ± 2.1 apoptotic cells at $IC_{50}$ concentration, which increased significantly to 67.4 ± 3.2 at 200 µg/mL. Similarly, *Mangifera indica* extract induced 38.3 ± 2.5 apoptotic cells at $IC_{50}$, rising to 72.5 ± 3.6 at higher concentration. The apoptotic ratio also increased from 0.31 ± 0.02 to 2.06 ± 0.15 for *Vitis vinifera* and from 0.62 ± 0.04 to 2.63 ± 0.18 for *Mangifera indica*, indicating enhanced apoptotic induction with increasing dose.

In MCF-7 cells, a comparable trend was observed. *Vitis vinifera* treatment produced 46.5 ± 2.8 apoptotic cells at $IC_{50}$ and 77.3 ± 3.5 at 200 µg/mL, while *Mangifera indica* showed 35.2 ± 2.4 and 69.5 ± 3.1 apoptotic cells at the respective concentrations. The apoptotic ratio increased from 0.86 ± 0.06 to 3.40 ± 0.20 for *Vitis vinifera* and from 0.54 ± 0.05 to 2.27 ± 0.16 for *Mangifera indica*. The higher apoptotic ratios (>1) observed at 200 µg/mL indicate a predominance of apoptotic cells over viable cells, confirming strong induction of programmed cell death.

Statistical analysis ($p < 0.05$) revealed that the increase in apoptosis at higher concentrations was significant compared to $IC_{50}$ levels. Overall, both seed protein extracts effectively induced apoptosis in HeLa and MCF-7 cells, with *Vitis vinifera* showing relatively higher activity in MCF-7 cells, while *Mangifera indica* exhibited notable effects in HeLa cells. These findings support the potential application of these plant-derived proteins as promising anticancer agents through apoptosis-mediated mechanisms (Table 6).

## DISCUSSION

The present study demonstrated that seeds of *Vitis vinifera* contain higher protein levels than *Mangifera indica*, consistent with earlier reports indicating the nutritional and biochemical richness of grape seeds (Shi *et al.*, 2003; Soong and Barlow, 2004). The higher protein yield in grape seeds may be attributed to the abundance of soluble storage proteins and efficient extraction under optimized buffer conditions.

The purification strategy involving ammonium sulfate precipitation, dialysis, and chromatography resulted in a progressive increase in specific activity, confirming effective enrichment of target proteins. The initial low specific activity in crude extracts and subsequent improvement through purification steps align with established protein purification principles (Scopes, 1994; Dixon and Webb, 1979). Chromatographic separation further enhanced purity, supporting its role as a high-resolution technique for protein isolation (Queiroz *et al.*, 2001).

Spectrophotometric analysis at 280 nm correlated well with Lowry-based protein estimation, validating the presence of aromatic amino acid-rich proteins, as described by Layne (1957). SDS-PAGE profiles revealed greater protein diversity in grape seed extract, with multiple bands indicating heterogeneous protein composition, whereas mango seed proteins showed comparatively fewer bands, suggesting lower complexity (Shewry *et al.*, 1995).

The protein extracts exhibited significant dose-dependent anti-proliferative activity against cancer cell lines, with HeLa cells showing greater sensitivity than MCF-7 cells. The reduction in cell viability observed in the MTT assay is consistent with mitochondrial dysfunction and metabolic inhibition (Mosmann, 1983). Morphological and fluorescence analyses confirmed apoptosis as the primary mode of cell death, evidenced by characteristic features such as membrane blebbing, chromatin condensation, and nuclear fragmentation (Ribble *et al.*, 2005).

Furthermore, the effectiveness of the protein extract at relatively moderate concentrations indicates its potential as a natural anticancer agent. The slightly lower sensitivity observed in MCF-7 cells may be due to their hormone-dependent nature and inherent resistance mechanisms, which have been reported to influence drug responsiveness.

## CONCLUSION

Proteins isolated from seeds of *Vitis vinifera* and *Mangifera indica* were successfully extracted, purified, and characterized. Grape seed extract exhibited higher protein content and greater molecular diversity compared to mango seed extract. The purified proteins showed significant hypoglycemic activity and dose-dependent anti-proliferative effects against cancer cell lines. Morphological analysis confirmed apoptosis as the primary mechanism of cytotoxicity. These findings highlight grape seed proteins as promising candidates for further development in anticancer applications. The present study concluded that grape seed proteins, in particular, represent a rich source of bioactive molecules with potential applications in anticancer therapies.

Further studies focusing on purification of individual protein fractions, structural characterization, and elucidation of molecular mechanisms are recommended to support their development as therapeutic agents.

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