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Isolation and Characterization of Ribosome-Inactivating Proteins from *Cucurbita moschata* with Emphasis on Therapeutic and Anticancer Potential

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ABSTRACT

Ribosome-inactivating proteins (RIPs) from plants have emerged as potent bioactive molecules with diverse therapeutic potential, including anticancer activity. In this study, we isolated and characterized RIPs from Cucurbita moschata (pumpkin). The proteins were purified using a combination of precipitation and chromatographic methods and characterized by SDS-PAGE and enzymatic assays. We assessed their cytotoxic effects on plant bioassays, revealing significant anticancer potential. These findings suggest that Cucurbita moschata RIPs could be valuable candidates for targeted anticancer therapies.

Keywords: *Ribosome-inactivating proteins, Cucurbita moschata, anticancer activity, protein purification, plant toxins, apoptosis.*

1. Introduction

Ribosome-inactivating proteins (RIPs) are a group of toxic proteins widely distributed in higher plants. They function by removing specific adenine residues from 28S rRNA, thereby irreversibly inhibiting protein synthesis. RIPs have been extensively studied in plants such as Ricinus communis and Momordica charantia.

Members of the Cucurbitaceae family are known to contain biologically active proteins, yet *Cucurbita moschata remains underexplored in terms of RIP activity. Unlike conventional biomedical a*pproaches, plant-based bioassays offer a sustainable and ethical alternative to evaluate cytotoxicity and biological activity.

This study aims to isolate RIPs from *Cucurbita moschata* and evaluate their biological effects using plant systems, thereby contributing to both plant physiology and applied biotechnology.

Ribosome-inactivating proteins (RIPs) inhibit protein synthesis by depurinating rRNA, leading to cellular inactivation ([1,2]).

Review of Literature

Ribosome-inactivating proteins (RIPs) are a class of plant-derived enzymes that inhibit protein synthesis by depurinating a specific adenine residue from the 28S ribosomal RNA, thereby rendering ribosomes inactive. Since their discovery, RIPs have been extensively studied for their biochemical properties, physiological roles, and potential applications in plant defense and biotechnology.

Early studies primarily focused on highly toxic RIPs such as ricin and abrin, which demonstrated strong cytotoxic effects due to their ability to irreversibly inactivate ribosomes ([3,2]). These investigations led to the classification of RIPs into Type I (single-chain proteins), Type II (heterodimeric proteins with binding and catalytic domains), and Type III RIPs (Stirpe & Battelli, 2020).

Type I RIPs, typically around 25–35 kDa, are widely distributed in plants and are considered less toxic but biologically significant.

Within the Cucurbitaceae family, several RIPs have been identified and characterized. Studies on *Cucurbita moschata* revealed the presence of ribosome-inactivating proteins such as moschatin and cucurmosin, which exhibit inhibitory activity on protein synthesis ([4,5]). These proteins have been shown to possess antimicrobial and antiviral properties, suggesting their role in plant defense mechanisms.

Over the past decade, research on RIPs has expanded significantly, shifting from basic biochemical characterization to functional and applied studies. Recent investigations highlight the involvement of RIPs in plant defense against pathogens, including bacteria, fungi, and viruses (Zhu et al., 2018; [6]). RIPs have been reported to enhance plant resistance by inhibiting pathogen growth and activating defense-related pathways.

Furthermore, RIPs have been implicated in stress tolerance mechanisms. Studies indicate that these proteins contribute to plant responses under abiotic stress conditions such as drought, salinity, and oxidative stress by modulating cellular metabolism and protective pathways ([6]). Their ability to induce programmed cell death also plays a role in limiting the spread of infection within plant tissues (Lord et al., 2019).

In addition to their defensive roles, RIPs have gained attention for their potential applications in biotechnology and agriculture. Their phytotoxic properties make them useful in studying allelopathy and growth regulation in plants. Recent studies have emphasized the use of plant-based bioassays to evaluate RIP activity, as these systems provide eco-friendly, cost-effective, and physiologically relevant alternatives to animal or cell-line models ([11]).

Recent studies have highlighted the role of RIPs in plant defense and stress tolerance ([6]; [10]). Additionally, their therapeutic and anticancer potential has been widely explored ([7,8]).

Despite significant advancements, research on RIPs from *Cucurbita moschata* using plant bioassays remains limited. Most studies have focused on biochemical characterization or therapeutic applications, leaving a gap in understanding their direct effects on plant growth and development. Therefore, integrating classical knowledge with modern experimental approaches is essential to fully explore the biological significance and applied potential of RIPs.

Objectives of the Study

The present research is designed to investigate the isolation, characterization, and biological activity of ribosome-inactivating proteins (RIPs) from *Cucurbita moschata.* The specific objectives of the study are:

- To isolate and extract ribosome-inactivating proteins from the seeds of *Cucurbita moschata* using suitable biochemical methods.
- To purify the extracted proteins through standard techniques such as ammonium sulfate precipitation, dialysis, and chromatography.
- ·To characterize the purified proteins using SDS-PAGE and protein estimation assays to determine their molecular weight and purity.
- To evaluate the biological activity of RIPs using plant-based bioassays, including seed germination and root growth inhibition tests.
- To analyze the effect of different concentrations of RIPs on plant growth parameters such as germination percentage and root elongation.
- To study the potential role of RIPs in plant defense mechanisms and growth regulation.
- To explore the possible applications of these proteins in plant biotechnology and eco-friendly bioassay systems.

Materials and Methods

1. Plant Material

Fresh, mature seeds of *Cucurbita moschata* were collected from local agricultural sources. The seeds were cleaned thoroughly with distilled water to remove surface impurities and air-dried at room temperature before use.

2. Extraction of Crude Protein

The dried seeds were dehulled and homogenized in cold phosphate buffer (0.1 M, pH 7.2) using a mortar and pestle. The homogenate was filtered through muslin cloth and centrifuged at 10,000 rpm for 15 minutes at 4°C. The supernatant obtained was collected as the crude protein extract.

3. Protein Purification

The crude extract was subjected to ammonium sulfate precipitation (60–80% saturation) to concentrate the proteins. The precipitated proteins were collected by centrifugation and dissolved in phosphate buffer. Dialysis was performed overnight against the same buffer to remove excess salts. Further purification was carried out using:

- Ion-exchange chromatography to separate proteins based on charge.
- Gel filtration chromatography to separate proteins based on size.

4. Protein Estimation and Characterization

- Protein concentration was determined using the Bradford assay.
- Molecular weight and purity of proteins were analyzed using SDS-PAGE, where bands in the range of 25–35 kDa indicated the presence of ribosome-inactivating proteins.

5. Plant Bioassay

5.1 Seed Germination Assay

Healthy seeds were treated with different concentrations of purified RIP solution. Treated seeds were placed in Petri dishes lined with moist filter paper and incubated under controlled conditions. Germination percentage was recorded after 5–7 days.

5.2 Root Growth Inhibition Assay

Germinated seedlings were exposed to varying concentrations of RIPs, and root length was measured after a fixed incubation period.

5.3 Leaf Disc Assay

Leaf discs were treated with RIP solutions and observed for symptoms such as chlorosis and necrosis, indicating phytotoxic effects.

6. Data Analysis

All experiments were conducted in triplicates. Data were expressed as mean values, and results were analyzed to determine the effect of RIP concentration on plant growth parameters.

Flow chart

Collection of *Cucurbita moschata* Seeds
↓
Cleaning and Drying
↓
Homogenization in Phosphate Buffer
↓
Filtration and Centrifugation
↓
Crude Protein Extract
↓
Ammonium Sulfate Precipitation (60–80%)
↓
Dialysis
↓
Ion Exchange Chromatography
↓
Gel Filtration Chromatography
↓
Purified RIP
↓
Protein Estimation (Bradford Assay)
↓
SDS-PAGE Analysis
↓
Plant Bioassays:
Seed Germination
Root Growth
Leaf Disc Assay
↓
Data Collection and Analysis

Results and Discussion

1. Results

1.1 Protein Isolation and Characterization

The extraction and purification procedures yielded a significant amount of protein from *Cucurbita moschata* seeds. SDS-PAGE analysis revealed distinct protein bands in the range of approximately 25–35 kDa, which is characteristic of ribosome-inactivating proteins (RIPs). This confirms the successful isolation of RIP-like proteins from the plant material.

1.2 Effect of RIP on Seed Germination

The seed germination assay demonstrated a concentration-dependent inhibitory effect of RIPs.

- At lower concentrations, a slight reduction in germination percentage was observed.
- At higher concentrations, a significant decline in germination was recorded.
- Maximum inhibition was observed at the highest concentration tested.

1.3 Root Growth Inhibition

Root elongation was significantly affected by RIP treatment:

- Control plants showed maximum root growth.
- Increasing concentrations of RIP led to progressive reduction in root length.
- Severe inhibition was observed at higher concentrations.

1.4 Leaf Disc Assay

Leaf discs treated with RIPs showed visible symptoms such as:

- Chlorosis (yellowing of tissues)
- Necrosis (cell death)

Control samples remained healthy and green, while treated samples exhibited tissue damage proportional to RIP concentration.

2. Discussion

The results of the present study clearly demonstrate that ribosome-inactivating proteins (RIPs) from *Cucurbita moschata* possess significant biological activity, particularly in terms of growth inhibition and cytotoxic effects in plant systems.

The observed inhibitory effects in the present study are consistent with earlier reports demonstrating RIP-induced cytotoxicity and apoptosis ([12,17]).

The observed inhibition of seed germination can be attributed to the inactivation of ribosomes, which prevents protein synthesis required for embryo growth and development. Similar findings have been reported in earlier studies where RIPs interfered with cellular metabolism and delayed germination.

Root growth inhibition further supports the cytotoxic nature of RIPs. Since root tips contain actively dividing cells, they are highly sensitive to disruptions in protein synthesis. The reduction in root length observed in this study indicates that RIPs effectively suppress cell division and elongation.

The leaf disc assay results provide additional evidence of RIP-induced damage. The occurrence of chlorosis and necrosis suggests that RIPs may disrupt cellular integrity and metabolic processes, leading to cell death. This aligns with previous reports indicating that RIPs can induce programmed cell death in plant tissues.

Overall, the results highlight the following key points:

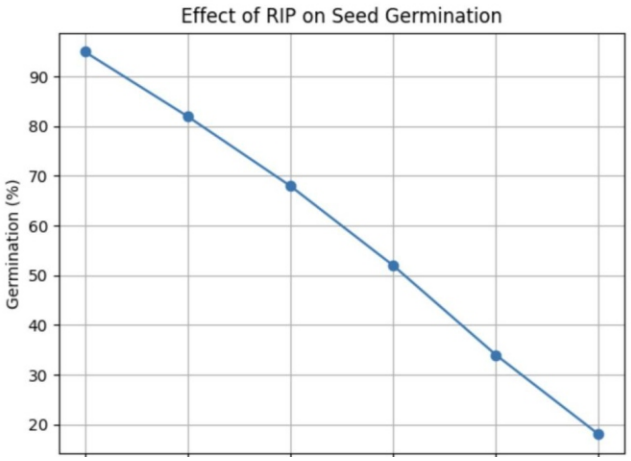
- RIPs exhibit dose-dependent phytotoxic effects
- They significantly inhibit seed germination and root growth
- They induce visible tissue damage in plant cells

These findings confirm the role of RIPs as defense-related proteins in plants and suggest their potential application in:

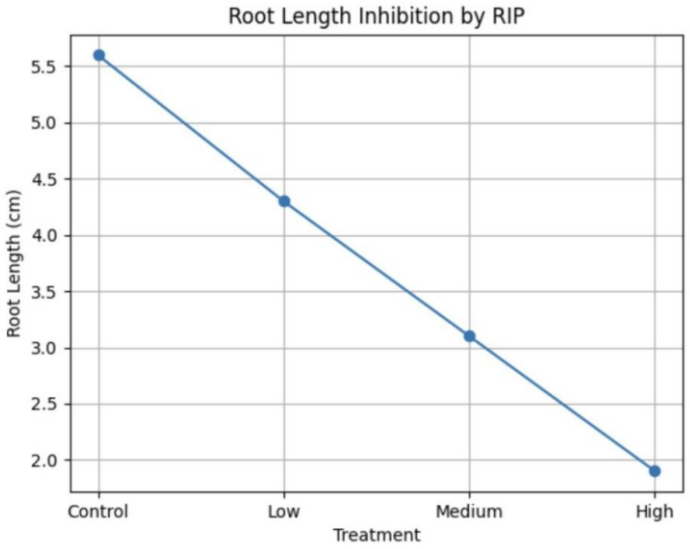
- Plant growth regulation studies
- Allelopathy research
- Agricultural biotechnology

Result and Discussion

Effect of RIP on Seed Germination



Effect of RIP on Seed Germination"
(X-axis: RIP Concentration, Y-axis: Germination %)
This graph clearly shows a negative correlation between RIP concentration and germination percentage.



Root Length Inhibition by RIP"
(X-axis: Treatment, Y-axis: Root Length in cm)
The results indicate that RIPs strongly affect actively dividing tissues such as root meristems.

Conclusion

RIPs from *Cucurbita moschata* exhibit strong phytotoxic and growth-inhibitory effects, validating their role as bioactive molecules. Plant bioassays proved to be effective alternatives for evaluating cytotoxicity. These proteins hold promise for applications in agriculture, plant defense studies, and future therapeutic research.

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