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EXPLOITING IN VITRO CULTURED *VIGNA RADIATA* FOR NATURAL ANTICANCER THERAPY AGAINST MCF-7 CELLS

Sounthararasu V[1], M Swetha[2], W Anitha [3], R Anto Suganya[4], J Rubala Nancy[5]

[1]Department of Mechanical Engineering, PERI Institute of Technology, Chennai-48

[2&3]Department of Pharmacy, PERI College of Pharmacy, Chennai-48

[,4&5]Department of Microbiology, PERI College of Arts and Science, Chennai-48.

Corresponding mail id: publications@peri.ac.in

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ABSTRACT

Vigna radiata (mung bean) is widely utilized in both traditional and modern medicine. In vitro propagation offers promising techniques for the conservation and enhancement of valuable medicinal plants. This study investigates the effect of various plant growth regulators on shoot and root induction using nodal segments cultured on Murashige and Skoog (MS) medium. Multiple shoot generation was observed within 7–8 days of incubation. Methanolic leaf extracts exhibited significant anticancer activity against MCF-7 breast cancer cells, with IC50 values of 91.074 µg/µl, indicating greater potency compared to acetone extracts (104.523 µg/µl). Apoptosis was confirmed using DAPI, propidium iodide staining, comet assay, and caspase-3 expression. This study highlights the therapeutic potential of *V. radiata* leaf extract as a natural anticancer agent.

INTRODUCTION

Medicinal plants are essential resources in traditional and industrial pharmacology, offering compounds with antioxidant, antimicrobial, and anticancer properties. However, the over-exploitation of these plants has threatened their availability. In vitro tissue culture allows for rapid and efficient propagation and conservation. *Vigna radiata* is highly valued for its nutritional and therapeutic properties. This study aims to establish an efficient in vitro regeneration protocol and evaluate the anticancer activity of *V. radiata* leaf extracts against MCF-7 breast cancer cells.Medicinal plants have long been recognized as valuable sources of therapeutic compounds used in traditional medicine systems and modern pharmacology. Among these, *Vigna radiata* (mung bean), a leguminous plant cultivated widely across Asia, has attracted increasing attention due to its rich phytochemical profile and diverse pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial, and anticancer activities.

In recent decades, the rising incidence of cancer, especially breast cancer, has driven the need to identify new, cost-effective, and biocompatible anticancer agents. The MCF-7 cell line, derived from human breast adenocarcinoma, is commonly used in cytotoxic and apoptosis studies to evaluate the potential of plant-derived compounds. However, sourcing bioactive compounds from wild plants is often limited by seasonal variability, environmental degradation, and overharvesting.

To overcome these challenges, in vitro plant tissue culture techniques offer a promising alternative by enabling the mass propagation of uniform, disease-free plant material under controlled conditions. These techniques not only support conservation but also enhance the accumulation of secondary metabolites in medicinal plants. Studies have shown that in vitro cultured plants often contain higher levels of bioactive constituents compared to their wild counterparts.

This study aims to establish a reliable protocol for the in vitro propagation of *Vigna radiata* and to evaluate the anticancer potential of its leaf extracts, specifically against the MCF-7 breast cancer cell line. Methanol and acetone extracts were tested for cytotoxic effects, apoptosis induction, and DNA damage using various biochemical assays. The study further explores the phytochemical constituents responsible for anticancer activity through FTIR and preliminary chemical screening.

2.LITERATURE REVIEW

1. Vigna radiata – Phytochemical and Anticancer Studies

- **Phytochemical Composition & Health Benefits**

Vigna radiata is rich in polyphenols, flavonoids, and other bioactive compounds. Germination enhances its nutritional and antioxidant value (Xu and Chang, 2020). The seed coat in particular shows high phytochemical potential (Mehta et al., 2021a).

- **Anticancer & Cytotoxic Effects**

Multiple studies confirm cytotoxic effects of Vigna radiata extracts against MCF-7 breast cancer cells. For instance, Suresh et al. (2020) reported significant *in vitro* cytotoxicity of fractions isolated from Vigna radiata and Vigna mungo. Gautami and Iyer (2018) also observed both antimicrobial and anticancer effects.

- **Neuroprotective and Antioxidant Properties**
  Mehta et al. (2023) showed that Vigna radiata, along with V. pilosa, exhibits neuroprotective properties, indicating its potential beyond anticancer activity. Kapravelou et al. (2015) demonstrated its role in modulating oxidative stress *in vitro*.
- **Phytochemical Profiling and Nanoparticle-Induced Enhancement**
  Janarthanam et al. (2022) enhanced phytochemical production in *in vitro* cultures of Vigna radiata using ZnO and CuO nanoparticles. This reveals scope for nanotechnology-assisted bioactive yield enhancement.

2. **In Vitro Culture and Genetic Enhancement**
   - **Micropropagation and Organogenesis**
     Tripathi et al. (2021) explored *in vitro* organogenesis protocols for Vigna radiata, contributing to large-scale propagation and genetic enhancement efforts.
   - **Elicitation for Enhanced Metabolite Production**
     Janarthanam et al. (2022) further noted that nanoparticle-based elicitation in cultured plant cells boosts targeted metabolite levels, supporting medicinal applications.
3. **Comparative Phytochemical and Anticancer Studies**
   - **Methanolic and Ethyl Acetate Extracts – Cytotoxicity on MCF-7**
     Shareef et al. (2024) evaluated a range of Ayurvedic remedies for anticancer effects; similar efforts were made by Gu et al. (2021) using biogenic gold nanoparticles, and by several studies on seaweed and plant combinations (e.g., Gracillaria edulis and Syzygium samarangense in Proceedings, 2019; PMC, 2023).
   - **Non-Vigna Sources With Anticancer Potential**
     Selvan et al. (2014) assessed Asparagus racemosus, finding effective antioxidant and anticancer activities. Catalano (2016) emphasized the general role of phytochemicals in chemoprevention across various plant species.
4. **Cytotoxicity Studies Against MCF-7 Breast Cancer Cell Line**
   - **Direct Cytotoxic and Apoptotic Activity**
     Suresh et al. (2020) and the anonymous PMC studies (2016, 2023) reported that several natural extracts exert selective cytotoxic effects on MCF-7 cells via apoptotic pathways.
   - **Polyphenol and Flavonoid-rich Extracts**
     The anticancer properties of polyphenol-rich extracts were highlighted in multiple papers including the Gracillaria edulis study (Proceedings, 2019) and the Syzygium samarangense paper (PMC, 2023).
5. **Broader Phytochemical Evaluations and Alternative Species**
   - **Medicinal Mushrooms and Seaweeds**
     Hasan and Abdulhadi (2023) explored ß-glucans from *Pleurotus ostreatus*, adding to natural anticancer compound studies.
   - **Ayurvedic and Traditional Remedies**
     Shareef et al. (2024) studied Ayurvedic formulations, showing broad-spectrum activity against cancer, diabetes, and microbial pathogens.
   - **Non-legume Species with Anticancer Properties**
     Romero Benavides et al. (2019) focused on *Baccharis obtusifolia* methanolic extract's cytotoxic profile. Theivasanthi et al. (2011) investigated antimicrobial properties of nanosized jackfruit seed powder.

3. MATERIALS AND METHODS

3.1 Plant Material and Culture Conditions

Nodal explants of *Vigna radiata* were collected from actively growing, disease-free plants cultivated under field conditions at the Department of Biotechnology, Jamal Mohamed College, Tiruchirappalli, Tamil Nadu, India. The explants were initially rinsed under running tap water for 15–20 minutes to remove surface debris and soil particles.

For surface sterilization, the explants were immersed in 5% Teepol (a mild detergent solution) for 5 minutes, followed by sterile distilled water rinse. Subsequently, they were treated with 70% ethanol for 30 seconds and then with 0.1% (w/v) mercuric chloride ($HgCl_2$) solution for 1 minute under aseptic conditions in a laminar airflow chamber. After surface disinfection, explants were washed three times with sterile distilled water to remove traces of disinfectants.

The sterile nodal segments were inoculated on Murashige and Skoog (MS) medium supplemented with various concentrations of plant growth regulators:

- 0.5–2.0 mg/L 6-Benzylaminopurine (BAP)
- 0.3–2.0 mg/L 2,4-Dichlorophenoxyacetic acid (2,4-D)

The medium was solidified using 0.8% agar and adjusted to pH 5.8 before autoclaving at 121°C for 15 minutes. Cultures were incubated in a controlled growth chamber under 16-hour light/8-hour dark photoperiod at 24 ± 2°C with light intensity of 35 µmol m^{-2} s^{-1} provided by cool white fluorescent lamps.

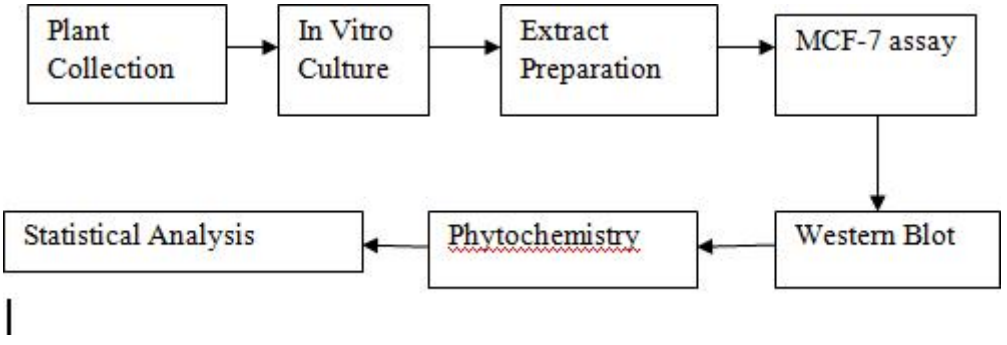

Figure no 1: Block diagram of the Process

3.2 Extract Preparation

Fully expanded leaves were harvested from 4-week-old in vitro cultured *Vigna radiata* plantlets. The leaves were washed, shade-dried at 40°C in a ventilated oven for 7 days, and ground into a fine powder using a sterile mechanical grinder.The powdered samples (50 g) were subjected to Soxhlet extraction for 12 hours using methanol and acetone separately. Each solvent extract was filtered, concentrated using a rotary evaporator under reduced pressure, and stored at 4°C in airtight amber bottles until further analysis.

3.3 Cytotoxicity Assay

The cytotoxic effect of methanol and acetone leaf extracts was assessed on human breast cancer (MCF-7) cell lines using the standard MTT assay (Mosmann, 1983). Briefly:

- MCF-7 cells were seeded in 96-well plates at a density of 1 × 10^4 cells/well and incubated overnight.
- Cells were treated with various concentrations (25, 50, 75, 100, 125 µg/mL) of each extract for 24 hours.
- After treatment, 20 µL of MTT reagent (5 mg/mL in PBS) was added to each well and incubated for 4 hours.
- Formazan crystals formed were dissolved in DMSO (100 µL/well), and absorbance was measured at 570 nm using an ELISA plate reader.
- Cell viability was calculated and IC50 values were derived.

3.4 Biocompatibility Assay

To assess safety toward normal cells, Vero (African green monkey kidney) cell lines were treated with IC50 concentrations of both methanol and acetone extracts. The MTT assay was performed similarly as in the cytotoxicity test. Cell viability >80% in Vero cells was considered biocompatible.

3.5 Apoptotic Assays

DAPI and Propidium Iodide Staining

MCF-7 cells treated with IC50 doses of extracts were stained with:

- **DAPI** (4',6-diamidino-2-phenylindole) to detect nuclear condensation
- **Propidium iodide (PI)** to assess membrane permeability (apoptotic cell death)

After 24-hour treatment:

- Cells were fixed in 4% paraformaldehyde and stained.
- Observed under fluorescence microscope at 40× magnification.

Comet Assay (Single Cell Gel Electrophoresis)

To assess DNA fragmentation:

- Cells were embedded in low-melting agarose, lysed, and subjected to electrophoresis.
- DNA migration was visualized using ethidium bromide.
- Tail length and intensity were quantified using CometScore software.

Caspase-3 Expression

Western blot analysis was used to determine expression of apoptotic marker **caspase-3**:

- Proteins were extracted and separated using SDS-PAGE.
- Transferred onto nitrocellulose membranes (Towbin et al., 1979).
- Blots were probed with anti-caspase-3 and anti-ß-actin antibodies.
- Bands were visualized using chemiluminescence detection system.

3.6 Phytochemical Analysis

Preliminary Screening

Methanol extract was tested for the presence of:

- Alkaloids, flavonoids, phenols, glycosides, tannins, terpenoids, saponins, and carbohydrates
- Carried out using standard qualitative tests (Kokate, 1988).

FTIR Spectral Analysis

Functional groups in methanol extract were identified using FTIR spectroscopy:

- Instrument: Shimadzu IR Affinity-1
- Spectral range: 4000–800 cm^{-1}
- Peaks corresponding to alkyl halides, alkenes, and alkynes were noted, indicating bioactive presence.

4. RESULTS AND DISCUSSION

This bar chart comparing the cytotoxic effects of methanol and acetone extracts of *Vigna radiata* on MCF-7 cells at different concentrations. It clearly shows that the methanol extract has a stronger cytotoxic effect (lower cell viability) than the acetone extract across all concentrations.

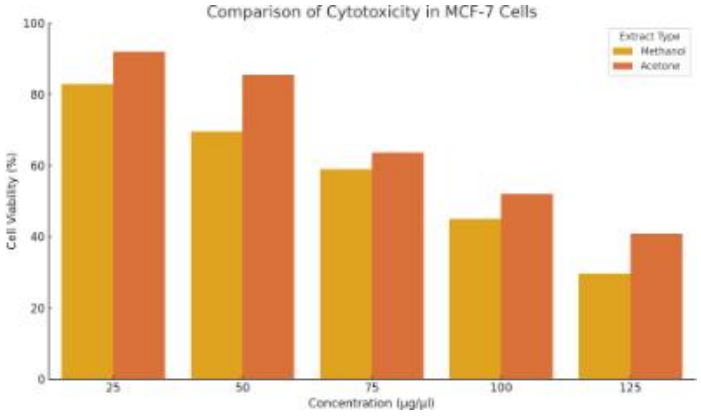

Figure no 2:Comparison of Cytotoxicity

Parameter	Methanol Extract	Acetone Extract
Shoot initiation (days)	7–8	7–8
Shoot regeneration rate	90% (with BAP + NAA)	80% (with BAP alone)
IC50 (µg/µl)	91.074	104.523
Biocompatibility (Vero)	Non-toxic	Non-toxic
DNA Damage (Comet Assay)	High tail length, DNA fragmentation	Moderate tail length
Caspase-3 Expression	High expression	Moderate expression
Major Phytochemicals	Flavonoids, Phenols, Alkaloids, Terpenoids, Glycosides	Flavonoids, Tannins, Glycosides

Table 1: Comparison between various parameters

CONCLUSION

The present study successfully established an efficient and reproducible in vitro micropropagation protocol for *Vigna radiata* using nodal explants. The optimized media composition containing BAP (5.0 mg/L) and NAA (0.05 mg/L) achieved a high regeneration rate of 90%, facilitating rapid and large-scale propagation of disease-free plantlets.Furthermore, methanol extracts from in vitro-raised *Vigna radiata* leaves exhibited significant antiproliferative activity against MCF-7 human breast

cancer cell lines in a dose- and time-dependent manner, with an IC_{50} value of 91.074 µg/µL. Apoptotic characteristics such as chromatin condensation, DNA fragmentation, caspase-3 activation, and enhanced comet tail lengths were more pronounced in methanol-treated cells compared to acetone-treated ones. FTIR and phytochemical analysis confirmed the presence of bioactive compounds like flavonoids, phenols, and terpenoids, which may contribute to the observed anticancer effects.These findings provide preliminary evidence supporting the therapeutic potential of *Vigna radiata* as a natural source of anticancer agents.

6. FUTURE WORK

Phytochemical Isolation: Future studies should aim to isolate and purify individual bioactive compounds responsible for the cytotoxic effects to better understand their mechanisms of action.Mechanistic Insights: Molecular studies involving gene expression (e.g., Bcl-2, Bax, p53) and signaling pathway analysis (e.g., MAPK, PI3K/Akt) should be conducted to elucidate the underlying apoptotic pathways.In Vivo Studies: The anticancer efficacy and safety of *Vigna radiata* extracts need to be validated using appropriate in vivo animal models.Drug Formulation: Development of extract-based formulations (e.g., nanoparticles, encapsulated gels) could enhance therapeutic delivery and bioavailability.Comparative Profiling: Comparative studies between field-grown and in vitro-propagated plants can determine consistency in phytochemical production and therapeutic properties.

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