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In vitro antiproliferative, antimitotic and cytotoxic studies on triphala: An Ayurvedic preparation

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Abstract
Triphala churna, a traditional Ayurvedic formulation composed of *Terminalia chebula*, *Terminalia bellerica*, and *Emblica officinalis* in equal ratios, is known for its therapeutic properties, including anti-inflammatory and antimicrobial effects. This study aimed to evaluate the anticancer potential of aqueous and ethanolic extracts of Triphala through in vitro antiproliferative, antimitotic, and cytotoxic assays. The extracts were prepared using distilled water and ethanol, then tested for antiproliferative activity using a yeast model and for antimitotic effects using *Allium cepa* root tips. Cytotoxicity was assessed via MTT assay on glioblastoma cell lines, using 12.5% and 25% concentrations of both extracts. The 25% ethanolic extract demonstrated the highest antiproliferative activity, showing 48% cell viability. It also exhibited the strongest antimitotic effect, reducing the mitotic index to 40%. The results suggest that ethanolic extracts of Triphala possess significant anticancer properties and could serve as promising candidates for future cancer therapies.

Keywords: Triphala, antiproliferative, antimitotic, cytotoxicity, glioblastoma, ethanolic extract

Introduction
Triphala, a classical Ayurvedic formulation, is described in traditional texts as a *Tridoshic Rasayana*, known for its balancing and rejuvenating effects on the body. According to the Ayurvedic Formulary of India (AFI), Triphala is composed of an equal mixture (1:1:1) of the dried fruits of *Terminalia chebula* Retz. (Haritaki), *Terminalia bellerica* Roxb. (Bibhitaki), and *Emblica officinalis* Gaertn. (Amalaki), and is one of the most widely used Ayurvedic preparations [1]. Traditional Ayurvedic literature recommends *Triphala churna* for the treatment of gastrointestinal and cardiovascular disorders, to promote ocular health, and for its anti-inflammatory properties [2, 4].
Previous research has reported the antimitotic potential of *Terminalia chebula* and the cytotoxic effects of *Emblica officinalis* [5, 7]. Furthermore, aqueous extracts of Triphala have been shown to influence microtubule assembly, thereby inhibiting the proliferation of cancer cells suggesting significant antimitotic activity [8]. These findings underscore the potential of this traditional formulation in modern therapeutic contexts and warrant further investigation.
The present study aims to evaluate the antiproliferative, antimitotic, and cytotoxic effects of aqueous and ethanolic extracts of *Triphala churna*. The extracts were analysed using established in vitro models to assess their potential as anticancer agents.

Materials and Methods
Collection of *Triphala Churna*
A reliable marketed product of *Triphala churna* was randomly selected from an Ayurvedic pharmacy. The product contained dried powders of *Terminalia chebula*, *Terminalia bellerica*, and *Emblica officinalis* in a 1:1:1 ratio. It was an authenticated, GMP-certified preparation.

Preparation of Extracts of *Triphala Churna* [9]
Aqueous extract: *Triphala churna* was extracted by cold infusion. The powder was suspended in distilled water at a 1:5 ratio and shaken intermittently using an orbital shaker for 48 hours. The mixture was filtered through Whatman filter paper, and the solvent was evaporated in a water bath maintained at 70-75°C to prevent overheating of phytochemicals.

The concentrated extract was used to prepare 12.5% and 25% dilutions.

Ethanolic extract: The same procedure was followed using ethanol instead of distilled water, resulting in 12.5% and 25% extracts.

Antiproliferative Study [10]

The antiproliferative activity was assessed using a yeast cell model.

- **Preparation of yeast inoculum:** Approximately 5 g of commercially available yeast was mixed with 100 mL sterilized nutrient broth and incubated at 37°C for 24 hours. One millilitre of this inoculum was diluted to 10 mL with sterile distilled water to obtain approximately 2.54×10^5 cells.
- **Preparation of potato dextrose broth (PDB):** 200 g of sliced potatoes were boiled in 1 L distilled water for 1 hour, filtered, and diluted to 1000 mL. Twenty grams of glucose were added, and the medium was autoclaved.

Cell viability assay: Test tubes containing 2.5 mL PDB, 1 mL of extract dilution, and 0.5 mL yeast inoculum were incubated at 37°C for 24 hours. Controls contained only PDB and yeast inoculum. Quercetin was used as a standard antiproliferative agent. After incubation, samples were stained with 0.1% methylene blue and observed under a microscope at 10× magnification. Viable (unstained) and dead (blue-stained) cells were counted in 16 haemocytometer squares. Cell viability (%) was calculated as:

$$Percentage\ cell\ viability = \frac{Total\ viable\ cells}{Total\ cells} \times 100$$

Antimitotic study [11]

- Collection of *Allium cepa*: Fresh onion bulbs (40–50 g) were obtained locally.
- Antimitotic assay: Outer dried layers were removed, and bulbs were placed in vessels with tap water to allow root growth of 3–4 cm. Roots were treated with aqueous and ethanolic extracts, with colchicine as a positive control and tap water as a negative control. After 72 hours, roots were harvested, fixed in acetic acid: ethanol (1:3), and treated with 1N HCl. Root tips were stained with acetocarmine and observed microscopically to count dividing and non-dividing cells. Mitotic index was calculated as:

$$Mitotic\ index = \frac{No:\ of\ dividing\ cells}{Total\ no\ of\ cells} \times 100$$

MTT Assay [12]

Glioblastoma cell lines were seeded at 5000 cells/well in 96-well plates and incubated overnight at 37°C with 5% CO_2. Cells were treated with Triphala extracts at concentrations of 10, 20, and 100 µg/mL and incubated for 24 hours. MTT reagent was added and incubated for 2–3 hours, after which the medium was removed, and formazan crystals were dissolved in solubilizing buffer. Absorbance was read at 570 nm using an ELISA reader. Cytotoxicity (%) was calculated as:

$$\%\ cytotoxicity = \frac{experimental\ absorbance\ [abs]\ 570\ nm\ of\ Exposed\ cells}{abs\ 570\ nm\ of\ unexposed\ cells} \times 100$$

Results and Discussions

1. Preparation of extracts of *Triphala churna:* The percentage yields of aqueous and ethanolic extracts were 47% and 39%, respectively.

2. Anti-proliferative study: Quercetin was used as the standard for comparison. Both aqueous and ethanolic extracts exhibited dose-dependent antiproliferative activity against yeast cells. The 25% ethanolic extract showed the highest activity with 48% cell viability at 0.125 mg/mL, while the aqueous extract was less active (cell viability ranging from 61.87% to 50.37%). Control samples showed 100% cell viability. These results, summarized in Table 1, demonstrate that the ethanolic extract of Triphala has significant antiproliferative effects. Yeast cells share key biological pathways with human cells, supporting the relevance of this model.

Table 1: Antiproliferative study

Extract	Average live cells	Average total cells	Viable cells/ml	& Cell viability
Aqueous Extract 12.5%	37	59.8	37×10^5	61.87
Aqueous Extract 25%	134	266	132×10^5	50.37
Ethanol Extract 12.5%	10.6	18	10.6×10^5	58.88
Ethanol Extract 25%	28.8	60	28×10^5	48
Standard	4.2	36.5	4.2×10^5	15.57

3. Antimitotic study: Colchicine was the positive control. All extracts showed a dose-dependent decrease in mitotic index compared to control (Table 2). The 25% ethanolic extract showed the greatest reduction with a mitotic index of 40%, while the 12.5% aqueous extract showed the least effect (80%). Reduced mitotic index indicates strong antimitotic activity, likely due to phytochemical interactions that delay interphase and inhibit progression to mitosis. Chromosomal abnormalities such as micronuclei, nucleolar disruption, binucleated cells, and chromosomal stickiness were observed, suggesting interference with DNA synthesis or spindle formation.

Table 2: Antimitotic activity

Extract	Total cells	Dividing cells	Non-dividing cells	Mitotic Index (%)
Aqueous Extract 12.5%	100	80	20	80
Aqueous Extract 25%	100	75	25	75
Ethanol Extract 12.5%	100	60	40	60
Ethanol Extract 25%	100	40	60	40
Standard	100	25	75	25

4. MTT Assay: The MTT assay on glioblastoma cell lines showed a concentration-dependent decrease in cell viability with both extracts (Table 3). Viability decreased below 100% at concentrations above 4 µg/mL. The ethanolic extract showed greater cytotoxicity than the aqueous extract. Glioblastoma is an aggressive brain tumor with poor prognosis, and these results suggest Triphala extracts have promising anticancer potential.

Table 3: MTT Assay

Dose (µg/mL)	Ethanol Extract (% Cell Viability)	Aqueous Extract (% Cell Viability)
4	90.21±3.12	93.45±1.89
20	85.66±2.33	89.02±2.24
100	72.84±1.85	75.89±2.17
500	61.55±2.61	67.56±1.57
5000	39.48±2.09	46.87±2.20

Conclusion

This study evaluated the cytotoxic effects of *Triphala churna* on glioblastoma cell lines. Both aqueous and ethanolic extracts exhibited dose-dependent cytotoxicity, supporting their potential use in cancer prevention and therapy. Antiproliferative and antimitotic activities further reinforce the therapeutic promise of this traditional formulation. Future studies should explore the cytotoxic effects in various cancer models and clarify the molecular mechanisms involved. Due to the complex nature of Ayurvedic formulations, determining bioavailability remains challenging, emphasizing the need for further pharmacokinetic and mechanistic research.

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