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Research Article

Assessing the Safety of Ethnobotanical Remedies: An *Allium cepa* Root Tip Study on Four Medicinal Plant Extracts

Chacko Suju Skaria*, Rejitha Lalitha Rajappan, Archana Sarah Biju

Department of Botany, Mar Ivanios College, Thiruvananthapuram, Kerala, INDIA.

ABSTRACT

Aim: This study evaluated the cytotoxic and genotoxic potential of leaf extracts from four ethnobotanically significant plants (*Trichopus zeylanicus, Naravelia alata, Cyclea peltata*, and *Smilax zeylanicus*) using the *Allium cepa* root tip assay to assess their safety for medicinal use. **Materials and Methods:** Aqueous leaf extracts were prepared at concentrations of 25%, 50%, 75%, and 100%. Onion root tips were exposed to each extract for 1 hr, fixed in Carnoy's fluid, hydrolyzed, and stained with acetocarmine for microscopic analysis. Mitotic Index (MI) and chromosomal aberrations were quantified to determine cytotoxicity and genotoxicity. **Results:** All extracts exhibited dose-dependent cytotoxicity, with MI decreasing significantly at higher concentrations. The 100% extract of *S. zeylanicus* showed the most severe suppression (MI=0.87%) and highest abnormality rate (99.1%). Chromosomal aberrations included stickiness, fragmentation, C-mitosis, bridge formation, and nuclear disintegration. *T. zeylanicus* and *N. alata* induced prominent stickiness and vacuolation, while *C. peltata* and *S. zeylanicus* caused spindle disturbances and clumping. **Conclusion:** The study confirms that these medicinal plants possess bioactive compounds with both therapeutic and genotoxic potential. The concentration-dependent effects highlight the need for careful dosage regulation in ethnomedicinal applications. Further phytochemical characterization is essential to isolate beneficial compounds while minimizing mutagenic risks.

Keywords: *Allium cepa* assay, Cytotoxicity, Genotoxicity, Medicinal plants, Mitotic abnormalities, Ethnobotany.

Correspondence:
Dr. Chacko Suju Skaria
Assistant Professor, Department of Botany, Mar Ivanios College, Thiruvananthapuram-695015, Kerala, INDIA.
Email: suju.skaria@mic.ac.in

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INTRODUCTION

Ethnobotany is the scientific study of the relationships between plants and people, particularly the traditional knowledge of indigenous cultures regarding the practical uses of regional flora.[1] Since the pioneering work of Richard Evans Schultes, often regarded as the father of ethnobotany, the field has evolved from merely documenting traditional plant uses to applying this knowledge in modern contexts-most notably in pharmaceutical development.[2] Despite the therapeutic potential of medicinal plants, many contain bioactive compounds that may exhibit toxicity, mutagenicity, carcinogenicity, or teratogenicity.[3] In African ethnomedicine, traditional healers employ a wide variety of herbs for disease treatment, many of which are presumed effective.[4,5] However, plants naturally synthesize toxic secondary metabolites as defense mechanisms against pathogens, herbivores, and environmental stressors, which can also adversely affect humans.[6] Consequently, assessing the cytotoxicity and genotoxicity of medicinal plants is essential to ensure their safe use.[7] Recent years have seen increasing reports of adverse effects associated with phytomedicines, underscoring the need for rigorous toxicological evaluation.[8-14]

The study of mitotic mechanisms is most effectively conducted in rapidly dividing plant tissues, such as root or shoot apical meristems.[15] Researchers frequently employ chemical agents that disrupt mitosis to investigate chromosomal behavior. One of the most widely used compounds is colchicine, an alkaloid derived from *Colchicum autumnale* L., known for its spindle-inhibiting properties, which leads to polyploidy and chromosomal aberrations.[16] However, due to its high cost and limited availability, alternative plant-derived mitotic inhibitors are of significant interest.[17] The *Allium cepa* (onion) root tip assay has become a standard model for assessing cytogenotoxicity, following Levan's demonstration of its sensitivity to spindle disturbances and chromosomal abnormalities induced by various agents.[18] Numerous studies have utilized this system to evaluate the effects of plant extracts on mitosis.[19-23]

The study focuses on four ethnobotanically significant plant species: *Trichopus zeylanicus, Cyclea peltata, Naravelia alata* (syn. *Narigamia alata*), and *Smilax zeylanicus*, collected from

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the biodiverse Kottoor forest. *Trichopus zeylanicus*, commonly known as "Arogyapacha," is a rare medicinal herb traditionally used in Ayurveda for its adaptogenic and anti-fatigue properties. *Cyclea peltata*, a climbing shrub, is utilized in folk medicine for its antipyretic and diuretic effects, with studies suggesting bioactive alkaloids contributing to its therapeutic potential. *Naravelia alata*, a woody climber, has been employed in traditional wound healing and anti-inflammatory treatments, though its cytotoxic effects remain understudied. Lastly, *Smilax zeylanicus*, a thorny vine, is widely used in Ayurveda and Siddha medicine for treating skin diseases, rheumatism, and venereal disorders, attributed to its rich phytochemical profile, including flavonoids and saponins.[24] Given their ethnomedicinal importance and limited scientific validation of their safety, these plants were selected for cytogenotoxicity assessment using the *Allium cepa* model.

MATERIALS AND METHODS

Plant Material Collection

Fresh leaves of *Naregamia alata, Cyclea peltata, Smilax zeylanica*, and *Trichopus zeylanicus* were collected from the Kottoor forest, Kerala, India. Traditional medicinal uses of these plants were documented through interviews with local tribal practitioners. Voucher specimens were deposited at Herbarium, Mar Ivanios College, Thiruvanathapuram for taxonomic authentication. The leaves were washed, shade-dried, and powdered for aqueous extraction.[25]

Preparation of Plant Extracts

Aqueous extracts were prepared by boiling 100 g of dried leaf powder in 500 mL of distilled water at 60°C for 2 hr. The mixture was filtered through Whatman No. 1 filter paper, and the supernatant was concentrated using a rotary evaporator at 40°C. The crude extract was stored at 4°C until further use.[26]

Allium cepa Root Tip Assay

Healthy onion bulbs (*Allium cepa*) of uniform size (2-3 cm diameter) were selected. Outer scales and old roots were removed, and bulbs were placed in sterilized soil for root initiation. After 72 hr, roots (1-2 cm long) were exposed to four concentrations (25%, 50%, 75%, and 100%) of each plant extract for 24 hr. A control group was maintained in double-distilled water.[27]

Fixation and Staining

Root tips (1 cm) were excised and immediately fixed in Carnoy's fluid I (3:1 ethanol:glacial acetic acid) for 24 hr to preserve cellular structure.[28] Fixed roots were hydrolyzed in 1 N HCl at 60°C for 15 min, rinsed with distilled water, and stained with 2% acetocarmine for chromosome visualization.[29]

Slide Preparation and Microscopic Analysis

Meristematic root tips (~1 mm) were squashed in acetocarmine under a coverslip. Slides were gently tapped to disperse cells, flattened using blotting paper, and sealed with wax. Mitotic stages were observed under a compound microscope (10× and 100× oil immersion). Images were captured using an image analyser.[30]

Data Analysis

Mitotic Index (MI) was calculated as:

$$\text{MI} = \frac{Number\ of\ dividing\ cells}{Total\ number\ of\ cells} \times 100$$

Abnormality Percentage was determined by:

$$\text{Abnormality \%} = \frac{\textbf{Number of abnormal cells}}{\textbf{Total number of cells}} \times 100$$

RESULTS

Cytotoxic Effects of Leaf Extracts on *Allium cepa* Root Tip Cells

The present study investigated the cytotoxic and genotoxic potential of four ethnobotanically significant plant extracts (*Trichopus zeylanicus, Naregamia alata, Cyclea peltata*, and *Smilax zeylanica*) using the *Allium cepa* root tip assay. The Mitotic Index (MI) and chromosomal aberrations were evaluated to assess the dose-dependent effects of the extracts.

Mitotic Index and Concentration-Dependent Inhibition

Treatment with aqueous leaf extracts significantly reduced the Mitotic Index (MI) in a concentration-dependent manner (Table 1). The highest suppression of cell division was observed at 100% concentration for all extracts.

Chromosomal Aberrations

The percentage of abnormal cells increased proportionally with extract concentration. The key abnormalities observed included:

Interphase Abnormalities

Vacuolation: Nuclei exhibited excessive vacuole formation, particularly at 75-100% concentrations (Figure 1). This may result from disrupted nucleic acid metabolism. Nuclear elongation and disintegration: Observed in *T. zeylanicus* and *N. alata* treatments, indicating severe cytotoxicity.

Prophase Abnormalities

Chromosome stickiness and fragmentation: Frequent in *S. zeylanicus* and *C. peltata* treatments (Figure 2), suggesting DNA damage or cross-linking.

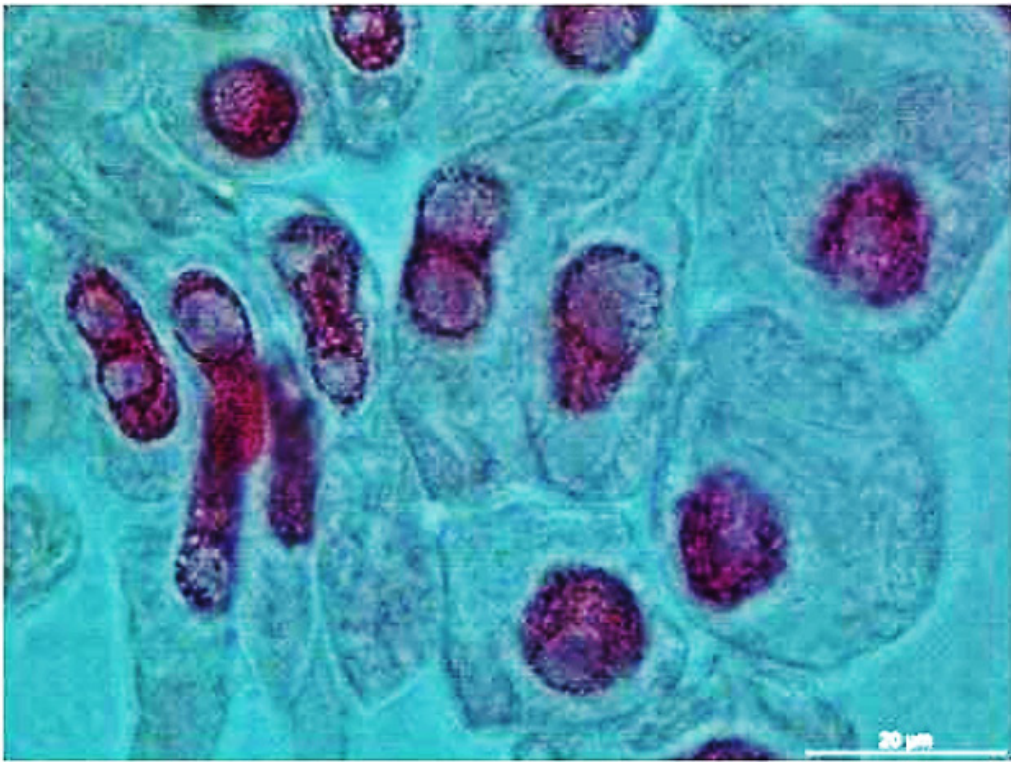

Figure 1: Vacuole Formation.

Table 1: Effect of leaf extracts on mitotic index.

Plants	Concentration	Treatment Time	Total No. Cells	No. of Dividing Cells	No. of Abnormal Cells	Mitotic Index	Percentage of Abnormalities
Trichopus zeylanicus	25%	1 Hr	985	250	735	25.38%	75%
	50%	1 Hr	1085	210	875	19.35%	81%
	75%	1 Hr	820	130	690	15.85%	84%
	100%	1 Hr	750	60	890	8%	92%
Cyclea peltata	25%	1 Hr	1830	497	833	37.36%	63%
	50%	1 Hr	1386	378	1008	27.27%	73%
	75%	1 Hr	1540	280	1260	18.18%	82%
	100%	1 Hr	1400	140	1260	10%	90%
Smilax zeylanicus	25%	1 Hr	1400	525	875	37.50%	63%
	50%	1 Hr	896	280	616	31.25%	69%
	75%	1 Hr	483	70	413	14.49%	86%
	100%	1 Hr	805	7	798	0.87%	99%
Naregamia alata	25%	1 Hr	1400	490	910	35%	65%
	50%	1 Hr	1764	3850	1414	19.84%	80%
	75%	1 Hr	1750	273	1477	15.60%	54%
	100%	1 Hr	1890	140	1750	7.40%	93%

Metaphase Abnormalities

Unoriented chromosomes and spindle defects: Clumped chromosomes ("sticky metaphase") were prominent in *T. zeylanicus* (Figure 3), likely due to depolymerization of spindle fibers.

Anaphase-Telophase Abnormalities

Chromosomal bridges and laggards: Caused by dicentric chromosomes or failed chromatid separation (Figure 4).

C-mitosis

Induced by *N. alata* and *C. peltata*, indicating spindle poisoning (Figure 5).

DISCUSSION

The *Allium cepa* root tip assay has been widely employed as a sensitive and reliable bioindicator for evaluating the cytotoxic and genotoxic potential of various chemical compounds and plant extracts. This test system offers several advantages, including clear chromosomal visibility, rapid cell division, and the ability to detect a wide range of chromosomal abnormalities. In the present study, we utilized this well-established model to assess the mitodepressive and clastogenic effects of leaf extracts from four medicinal plants. This research demonstrates that leaf extracts of *Trichopus zeylanicus, Narigamia alata, Cyclea peltata*, and *Smilax zeylanicus* induce significant mitotic abnormalities in *Allium cepa* root tip cells. These abnormalities include chromosomal stickiness, vacuolated cells, bi- and trinucleated cells, disturbed metaphase and anaphase, and eventual cell lysis at higher concentrations. The mitotic index (MI) was found to be inversely proportional to the extract concentration, indicating a dose-dependent inhibitory effect on cell division. These findings align with previous reports on the mutagenic potential of various plant extracts, including *Euphorbia hirta, Ocimum gratissimum, Morinda lucida*, and *Solanum nigrum*.[31-33]

The reduction in MI suggests either cytotoxic effects or a delay in cell proliferation kinetics.[34] A MI below 22% is considered lethal,[35] and in our study, higher concentrations (100%) of *S. zeylanicus* extract reduced MI to 0.869%, with 99.1% abnormality, indicating severe cytotoxicity. Similar observations were reported for *Terminalia chebula* and *Catharanthus roseus*, where bioactive compounds inhibited mitosis by binding to tubulin and disrupting spindle formation.[36,37] The disintegration of nuclei and cell wall lysis at high concentrations may result from direct interactions between phytochemicals and DNA-associated proteins or spindle apparatus.[38]

Despite their medicinal benefits, many plants exhibit cytotoxic effects at certain concentrations. *T. zeylanicus*, known for enhancing male reproductive performance in mice[39] and anti-ulcer activity in rats,[40] also induced chromosomal aberrations in A. cepa. Similarly, N. alata, valued for its antibacterial properties[41] and use in treating skin diseases,[42] exhibited dose-dependent cytotoxicity in our study. *C. peltata*, despite its diuretic and antidiabetic effects,[43,44] contains bisbenzylisoquinoline alkaloids with reported cytotoxicity against tumor cell lines.[45] Likewise, *S. zeylanica*, a potent antioxidant and substitute for Ayurvedic Chopacheeni,[46,47] showed extreme growth inhibition and lethality in *A. cepa* at higher concentrations, corroborating previous findings on its allelopathic effects.[48]

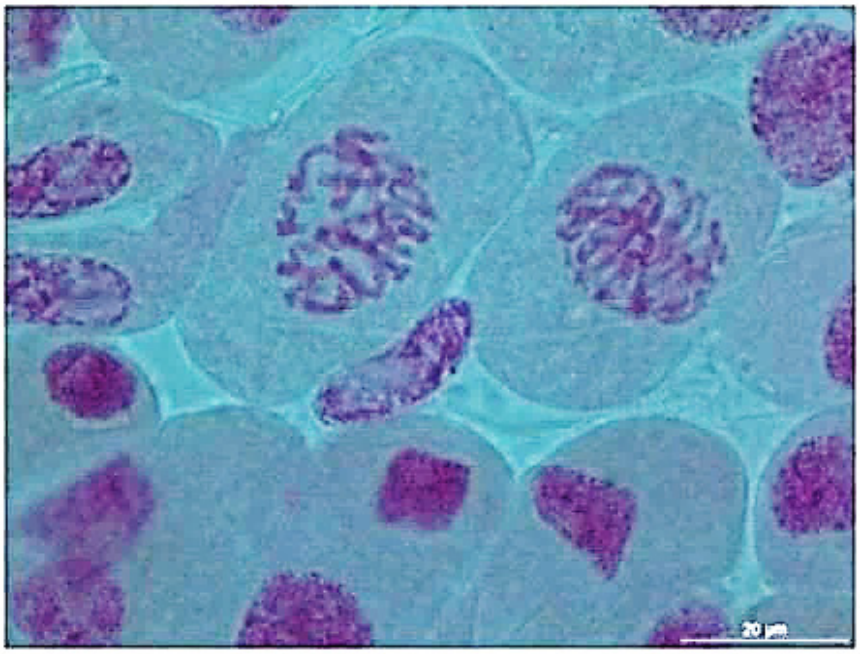

Figure 2: Fragmentation of Nucleus.

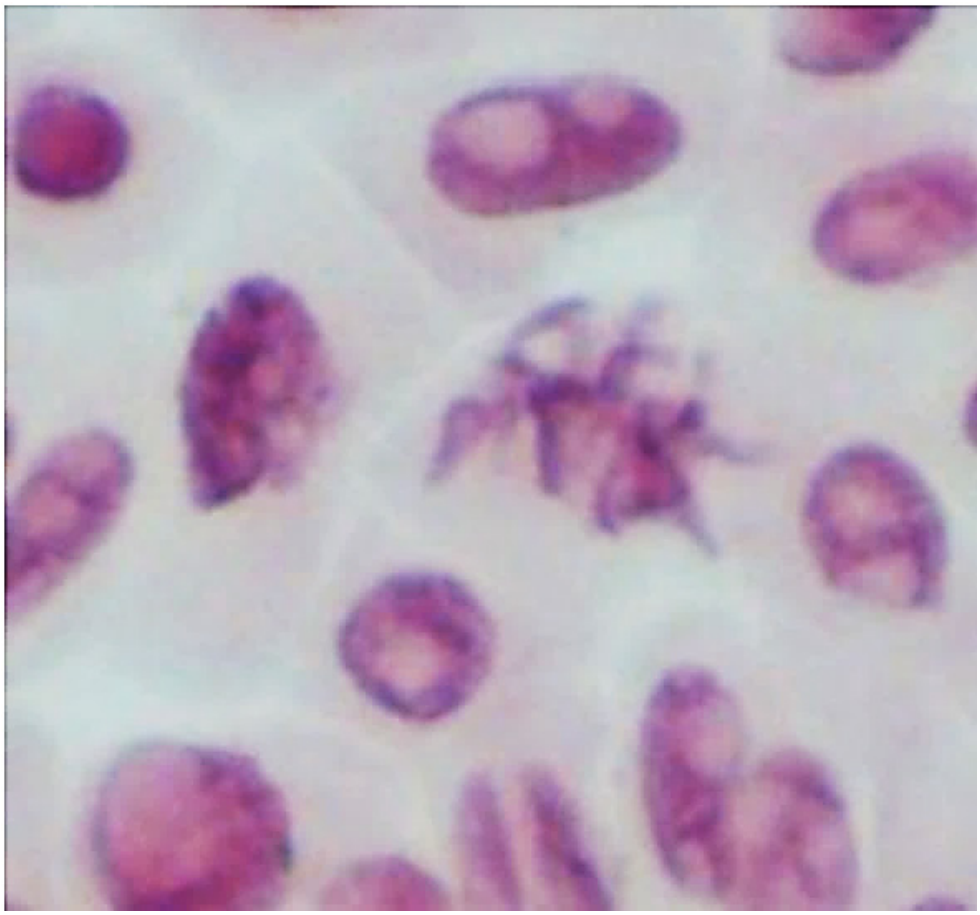

Figure 3: Metaphase abnormalities.

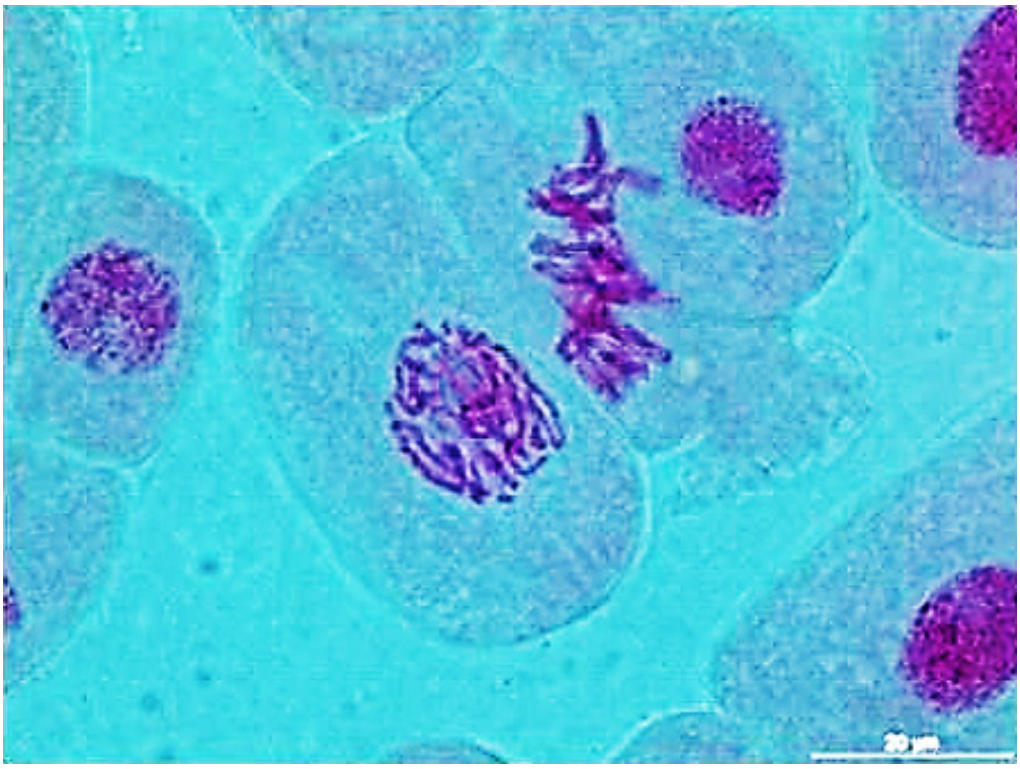

Figure 4: Chromosomal Bridges.

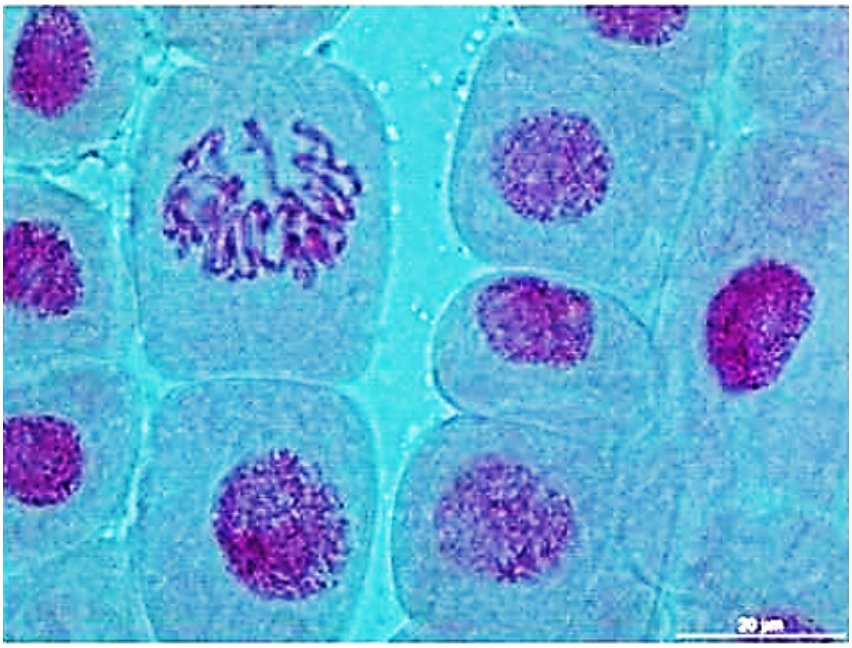

Figure 5: C-Mitosis.

CONCLUSION

The leaf extracts of *T. zeylanicus, N. alata, C. peltata*, and *S. zeylanicus* showed concentration-dependent cytotoxic effects on *A. cepa* root tip cells, with higher concentrations (25-100%) causing greater mitotic inhibition and chromosomal abnormalities like stickiness, fragmentation, and bridge formation. These findings reveal the plants' dual potential-while possessing bioactive compounds of medicinal value, they may also exhibit mutagenic effects. The dose-responsive nature of these effects underscores the need for careful phytochemical analysis before therapeutic application. The *A. cepa* test reliably indicates these genotoxic effects, suggesting similar caution should be exercised in mammalian systems. Further research should focus on isolating and characterizing the active compounds responsible for both beneficial and harmful effects.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

MI: Mitotic Index; **DNA:** Deoxyribonucleic Acid; **HCl:** Hydrochloric Acid; **HIV:** Human Immunodeficiency Virus; **Hr:** Hour; **mL:** Milliliter; **g:** Gram; **N:** Normal; **No:** Number; **cm:** Centimeter; **mm:** Millimeter; **°C:** Degree Celsius.

SUMMARY

While these plants possess significant therapeutic potential, their cytotoxic effects warrant caution in medicinal applications. Further studies should isolate active compounds and assess their genotoxic and anticancer properties to balance efficacy and safety.

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