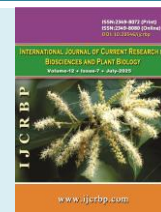




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## Cytotoxicity Screening of Selected Antimalarial Botanicals in Ekiti State, Nigeria

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Article Info	Abstract
<b>Keywords:</b> Botanicals, cytotoxic, morphotoxic, mitotic, aberration, phytochemical, bioassay	This study investigates the cytotoxic and genotoxic effects of <i>Mangifera indica</i> and <i>Blighia sapida</i> leaf extracts on <i>Allium cepa</i> roots, focusing on root length, root number, mitotic index (MI), and phase index. The study aims to evaluate the potential of these plant extracts to inhibit root growth and disrupt cellular division processes. <i>Allium cepa</i> roots were treated with various concentrations of the extracts (5%, 10%, 15%, 25%, 50%, 75% and 100%), and the resulting changes in root length, number, and mitotic activity were recorded. A concentration-dependent decrease in root length and number was observed, indicating significant growth inhibition and cytotoxicity at higher extract concentrations. The mitotic index and phase index also decreased with increasing concentrations of the extracts (72%-19% <i>Mangifera indica</i> , 72%-20% <i>Blighia sapida</i> ), suggesting that the extracts hinder cell division by interfering with the mitotic spindle and causing cell cycle arrest. Chromosomal aberrations, including anaphase bridges and sticky chromosomes, were noted, reflecting the genotoxic potential of the phytochemicals present in the extracts. The study identified key bioactive compounds such as quercetin, mangiferin, and scopoletin, which are known to disrupt microtubule dynamics, inhibit cell proliferation, and induce apoptosis. These findings underscore the importance of comprehensive toxicity testing in evaluating the safety and efficacy of plant extracts for medicinal or agricultural applications. The significant cytotoxic and genotoxic effects observed in this study highlight the potential risks associated with the use of <i>Mangifera indica</i> and <i>Blighia sapida</i> extracts, emphasizing the need for careful consideration in their application. This study contributes to the growing body of literature on the biological impacts of plant extracts, providing valuable insights into their mechanisms of action at the cellular level.
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### Introduction

The widespread use of botanical remedies for treating

diseases, particularly in traditional medicine, has drawn significant scientific attention due to their potential therapeutic benefits (Dao et al., 2021). Among these,

antimalarial botanicals have garnered interest as alternatives to synthetic drugs, which often come with significant side effects and the risk of resistance development by malaria parasites (Rumisha et al., 2019). However, while the efficacy of these plants is often highlighted, their cytotoxic effects remain a critical concern, necessitating thorough investigation. This study focuses on evaluating the cytotoxicity and cytological impacts of two widely used antimalarial botanicals: Mango (*Mangifera indica*) and Ackee Apple (*Blighia sapida*), using the *Allium cepa* bioassay. *Mangifera indica* (Mango), a plant native to South Asia but widely cultivated in tropical and subtropical regions, has been traditionally used not only for its nutritional value but also for its medicinal properties.

The leaves, bark, and seeds of Mango are reported to possess antimalarial, anti-inflammatory, and antimicrobial properties, making it a popular choice in traditional medicine (Royal Botanic Garden, 2021). Similarly, *Blighia sapida* (Ackee Apple), primarily found in West Africa and the Caribbean, is known for its use in various folk remedies (Olayinka et al., 2023). The seeds and arils of Ackee Apple have been utilized for their purported antimalarial, analgesic, and anti-inflammatory effects (Olayinka et al., 2023). Despite their widespread use, there is limited scientific data on the cytotoxicity and cytological effects of these plants, which raises concerns about their safety as therapeutic agents.

The *Allium cepa* (onion) bioassay is a widely recognized method for assessing cytotoxicity and genotoxicity in plants [3, 4]. This assay is particularly valuable for its simplicity, cost-effectiveness, and ability to provide insights into cellular mechanisms such as mitosis, chromosomal aberrations, and root growth inhibition (Ihegboro et al., 2020). By employing the *Allium cepa* bioassay, this study aims to assess the cytotoxic effects of Mango and Ackee Apple on root growth, mitotic index, chromosomal aberrations, and phase indices. These parameters are critical indicators of the plants' potential to cause cellular damage, which could have implications for their safety as antimalarial agents. The cytotoxicity of plant extracts is closely linked to their chemical constituents, which can interact with cellular components to induce toxicity or therapeutic effects. In the context of malaria treatment, cytotoxicity can be a double-edged sword (Boumaza et al., 2016). On one hand, cytotoxic compounds might target the malaria parasite effectively; on the other hand, they might also pose risks to the host's cells (Asanga et

al., 2023). Okaiyeto and Oguntibeju (2021) discuss the adverse effects and cytotoxic potentials of African herbal medicines, emphasizing the need for careful evaluation of these plant-based remedies before therapeutic use (Okaiyeto and Oguntibeju, 2021). Pathiratne et al., (2015) demonstrate the efficacy of the *Allium cepa* test system for detecting cytotoxicity and genotoxicity in industrial effluents, reinforcing the model's value in environmental monitoring (Pathiratne et al., 2015). Celik and Aslantruk (2020) explore the cytotoxic and genotoxic effects of *Inula viscosa* leaf extracts on *Allium cepa*, highlighting the plant's potential bioactivity and the importance of toxicity testing in assessing the safety of herbal extracts (Celik and Astranluk, 2020). Therefore, understanding the cytotoxic profile of antimalarial botanicals is crucial for their safe use and development into therapeutic agents.

This study is particularly significant given the global burden of malaria and the ongoing search for effective, affordable, and safe treatments. As resistance to conventional antimalarial drugs continues to rise, the exploration of plant-based alternatives becomes increasingly important. However, for these alternatives to be viable, they must not only be effective but also safe. The findings of this research will contribute to the growing body of knowledge on the cytotoxic effects of medicinal plants, specifically focusing on Mango and Ackee Apple, and will provide insights into their potential risks and benefits as antimalarial agents.

## Materials and Methods

### Study Area and Duration

This study was conducted between November 2023 and March 2024 in Oye-Ekiti, Ekiti State, Nigeria. Oye-Ekiti, situated at latitude 7.79°N and longitude 5.33°E, has an approximate population density of 206,300 and is predominantly inhabited by the Yoruba ethnic group. The economy of the region is primarily based on agriculture. The materials used for the study is presented in Table I.

### Selection of Plant Material

An ethnobotanical survey was carried out in 10 local governments of Ekiti State, Nigeria. 100 Questionnaire on "The Usage and Administration of Medicinal Plants in Ekiti State" was administered to the respondents which includes those who are vast in the knowledge and usage of traditional medicine. A total of 10 plants were

reported from the questionnaire to be broadly used in the treatment of malaria in Ekiti. Two out of the 4 most used with highest perceived efficacy was selected for the Cytotoxicity screening, namely; *Blighia sapida* (K.D. Koenig) and *Mangifera indica* L. The result of the ethnobotanical survey is shown in Table II.

### Cytotoxicity Testing

The cytotoxicity and morphotoxicity evaluation were carried out according to Celik and Astranluk (2020) and Leme and Marin-Morales (2020), with little adjustments to align to the research aim.

### Macroscopic Evaluation

Cytotoxicity testing of the selected botanicals was conducted using the *Allium cepa* bioassay, a widely accepted method for assessing the potential toxic effects of substances. The bioassay involved growing onion (*Allium cepa*) bulbs in various concentrations of botanical extracts to evaluate their impact on root growth. The powdered leaf extracts of Ackee Apple (*Blighia sapida*) and Mango (*Mangifera indica*) were used for this test. Each extract was dissolved in water to prepare solutions at concentrations of 5%, 10%, 15%, 25%, 50%, 75%, and 100%. A control group was maintained with tap water. Onion bulbs were placed in 100ml plastic cups filled with the prepared extract solutions. The bases of the bulbs were immersed in the solutions, while the bulbs themselves were kept in the dark to avoid photosynthesis, which could influence root growth. Each concentration was tested in triplicate to ensure reliable results. The bulbs were exposed to the test solutions for 96 hours. During this period, the solutions were replaced with fresh ones every 24 hours to maintain the effectiveness of the treatment. At the end of the exposure period, three bulbs exhibiting the best root growth from each concentration were selected for further analysis. The macroscopic evaluation focused on several key parameters: root length, root number, and root condition. Measurements of root length were taken with a meter rule, while the number of roots per bulb was counted. Root condition was assessed for visual changes, including discoloration, lesions, necrosis, and other abnormalities. The percentage of root growth inhibition was calculated to quantify the toxic effect of the extracts. The formula used for this calculation was:

### Microscopic Evaluation

For a detailed understanding of the cytotoxic effects,

microscopic evaluation was performed to assess chromosomal integrity and mitotic activity. This involved examining the root tips of the onions under a light microscope. After the macroscopic evaluation, root tips from the bulbs exposed to different concentrations of extracts and the control were fixed in Carnoy's solution (ethanol and glacial acetic acid). This fixative preserves the cellular structure by rapidly killing the cells and denaturing proteins, which helps in maintaining chromosomal integrity. The fixed root tips were stored at 4°C for 24 hours before processing. The root tips were treated with 1N hydrochloric acid and heated for 5 minutes to soften the tissue (Leme and Marin-Morales, 2020). They were then rinsed with distilled water, placed on microscopic slides, and stained with 2% acetocarmine. This stain helps visualize the chromosomes under the microscope. Cover slips were carefully placed over the stained samples, and excess stain was blotted out to minimize artifacts. The prepared slides were examined using a Light Microscope at  $\times 40$  magnification. A total of 500 cells from three scorable slides per sample were observed for various mitotic stages and chromosomal abnormalities. Key metrics included the mitotic index, which measures the proportion of cells undergoing mitosis, and the number of chromosomal aberrations.

### Data Analysis and Parameters Calculated

Data analysis was conducted using R Studio to interpret the results from both macroscopic and microscopic evaluations. One-Way ANOVA followed by Tukey's Honestly Significant Difference (HSD) test was employed to compare the effects of different extract concentrations and the control group. This analysis assessed whether differences in root growth, mitotic index, and chromosomal abnormalities were statistically significant.

- 1. Root Growth Inhibition:** The mean root length was compared between treated and control groups to calculate the percentage of root growth inhibition.
- 2. Mitotic Index:** The proportion of dividing cells was calculated using the formula:  $\text{Mitotic Index} = (\text{Number of Dividing Cells} / \text{Total Number of Cells}) \times 100$
- 3. Chromosomal Aberrations:** The percentage of aberrant cells was determined with:  $\text{Percentage Abnormal Cells} = (\text{Number of Aberrant Cells} / \text{Total Number of Cells}) \times 100$

## Presentation of Results

The results were summarized in tables and charts to provide a clear visual representation of the effects of each extract concentration. The macroscopic results highlighted the degree of root growth inhibition, while the microscopic analysis provided insights into the cellular and chromosomal impacts of the extracts. Statistical significance was denoted where p-values were less than 0.05.

## Macroscopic Analysis Result

Table III shows the effect of different concentrations of *Mangifera indica* in the root inhibition of *A. cepa*. As presented in the table, the root length and the root number decreased as the concentration increase. As observed in the table, 5%, 10% and 15% are not significantly different for the root length and but 15% is significantly different in the root number. 25% and 50% are significantly different, while 75% and 100% are not significantly different for root length (but significantly different for the root number). It was also observed from the % root growth that all the concentrations are significantly different. The root length decreased from the control from 4.2cm to 0.6cm, number of roots 56.67 to 8, and % root growth inhibition 100% to 4.17%. Table IV shows the effect of different concentrations of *Blighia sapida* on the root inhibition of *A. cepa* root. According to the result, 10%, 15% and 25% concentration are not significantly different in the root length, but significantly different for the number of roots. For the % root growth inhibition, only 25% and 50% are not significantly different from each other. The root length was observed to decrease from 4.33 cm for control to 0.53% (100%), while the number of roots decreased from 55 to 6.33 (control to 100%). The % root growth inhibition significantly decreased from 100% for control to 5.17% at 100% concentration. Figure I shows the compared effect of different concentrations of *Blighia sapida* and *Mangifera indica* in the root number of *Allium cepa* root. This further buttress the details presented in Table II and III, providing a comparison of both plants. While Figure II shows the compared effect of different concentrations of *Blighia sapida* and *Mangifera indica* in the root length of *Allium cepa* root. It shows the comparative chart of the root length performance of both plants on the Allium root morphology.

## Microscopic Analysis Result

Table V present the effect of crude leave extract of

*Mangifera indica* on the mitotic index and the percentage chromosomal aberration of *A. cepa* root. As presented in the table, the mitotic index decreased as the concentration increase. The result of the mitotic index is significantly different for all of the concentration (5%, 10%, 15%, 25%, 50%, 75% and 100%). The same was observed for the % mitotic inhibition for all the concentrations.

Significant decrease was also noticed for the phase index of all the concentration (Prophase, Metaphase, Anaphase, and Telophase). Table VI present the effect of crude leave extract of *Blighia sapida* on the mitotic index and the percentage chromosomal aberration of *A. cepa* root. As shown in the table, the mitotic index decreased as the concentration increase. The result of the mitotic index for all the concentrations is significantly different from each other (54%, 48%, 44%, 38%, 33%, 26% and 20%). The same was observed for the % mitotic inhibition for all the concentrations. Significant decrease was also noticed for the phase index of all the concentration (Prophase, Metaphase, Anaphase, and Telophase).

Table VII shows the effect of crude leaf extract *Mangifera indica* on the percentage chromosomal aberration of *A. cepa* root. According to the table below, eight different aberration was observed in the Allium root tip across the different concentrations. In 5%, 10%, and 15% concentration, no chromosomal aberration was observed. At 25%, nuclear lesion and vagrant chromosome was observed. For the 50% concentration, the observed chromosomal aberrations include, micronuclei, stickiness, nuclear lesion, intertwined chromosome and vagrant chromosome. For the 75% concentration, only micronuclei were not observed among the eight aberrations, while only intertwined chromosome was not observed in the 100% concentration of the Allium root.

Table VIII shows the effect of crude leaf extract *Blighia sapida* on the percentage chromosomal aberration of *A. cepa* root. According to the table below, eight different aberration was observed in the Allium root tip across the different concentrations. In 5% and 10%, concentration, no chromosomal aberration was observed. At 15%, giant chromosome, nuclear lesion and vagrant chromosome was observed. For the 25% concentration, the observed chromosomal aberrations include, micronuclei, giant chromosome, stickiness, nuclear lesion, intertwined chromosome and vagrant chromosome. For the 50% concentration, only vagrant



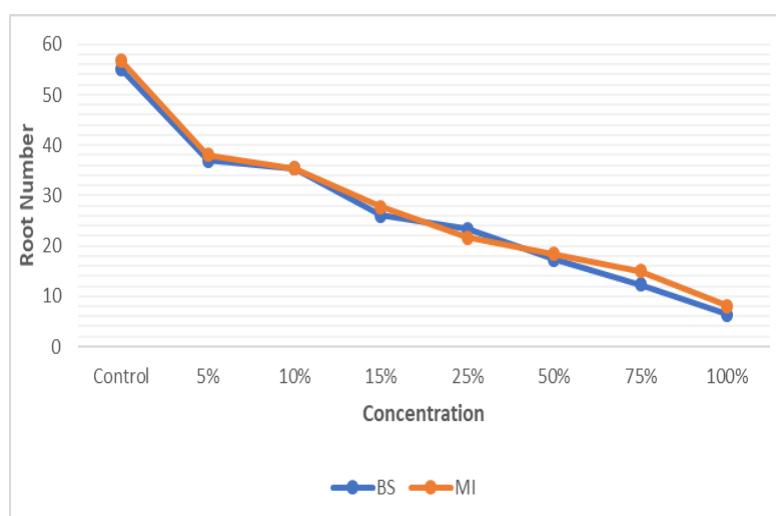
chromosome, blebbed chromosome and multinucleated cells were not observed were not observed among the eight aberrations. For the 75%, only vagrant was not observed, while every of the eight aberrations were observed in the 100% concentration of the *Allium cepa* root. Figure III shows the compared effect of the leaf extract of *Mangifera indica* and *Blighia sapida* on the number of aberrant cells of *A. cepa* root. From the figure, *Blighia sapida* has more severe effect compared to *Mangifera indica*. The effect became visible from 15% to 100%, though a similar effect was noticed at 50% and 75%. While Figure IV shows the compared effect of leaf extract of *Mangifera indica* and *Blighia sapida* on the percentage of aberrations of *A. cepa* root. The highest percentage of aberration was observed to increase as the concentration increased for both plants. However, the severity was more for *Blighia sapida* than *Mangifera indica*.

## Results and Discussion

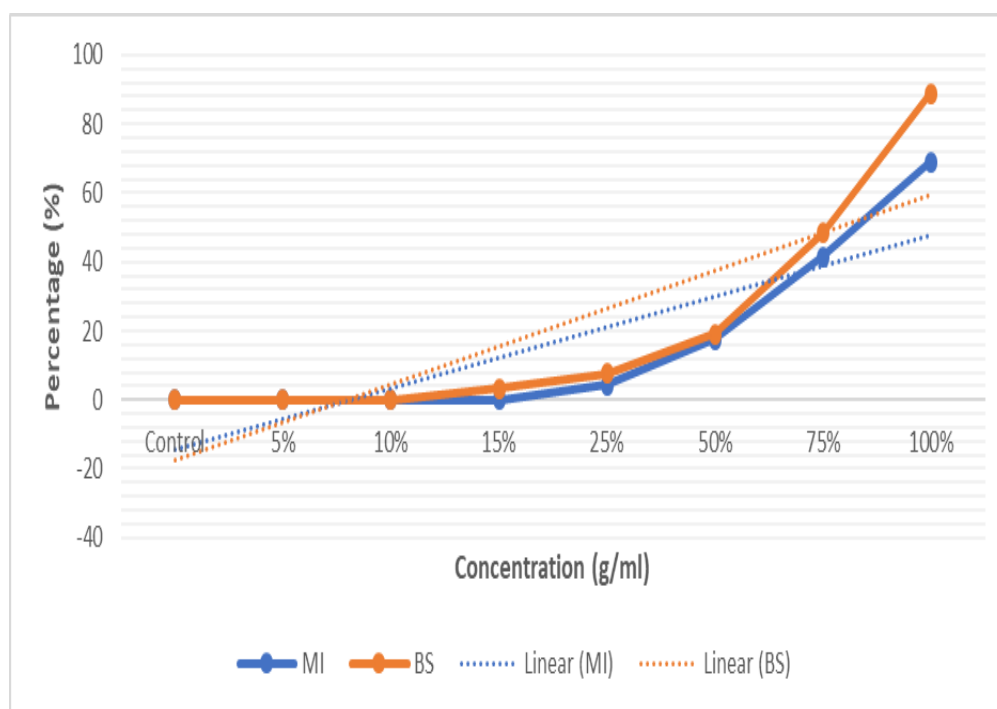
The root length and number are essential indicators of the health and viability of *Allium cepa* roots exposed to plant extracts (Leme and Marin-Morales, 2020). Changes in these parameters can reflect the cytotoxic, genotoxic, and growth-regulating effects of the phytochemicals present in the extracts. The root length and number are sensitive indicators of growth inhibition and overall root health. A decrease in root length and number indicates growth inhibition, while an increase may suggest root proliferation or abnormal growth patterns. Adekola et al., (2021) observed a concentration-dependent decrease in root length and

number in *Allium cepa* roots treated with ethanol and ethyl acetate extracts of *Blighia sapida*, indicating growth inhibition due to the cytotoxic effects of the extracts. The effect of plant extracts on root length and number, as well as root condition in *Allium cepa* roots, provides valuable insights into the cytotoxic, genotoxic, and growth-regulating properties of the phytochemicals present in the extracts (Navarro et al., 2019). Changes in root morphology, such as inhibition of growth, swelling, necrosis, lesions, curling, and tumor formation, reflect the complex interactions between the plant compounds and cellular processes in the root meristem (Khakdan and Piri, 2012). Understanding these effects is crucial for evaluating the safety and efficacy of plant extracts for medicinal or agricultural purposes and underscores the importance of comprehensive toxicity testing in plant-based research.

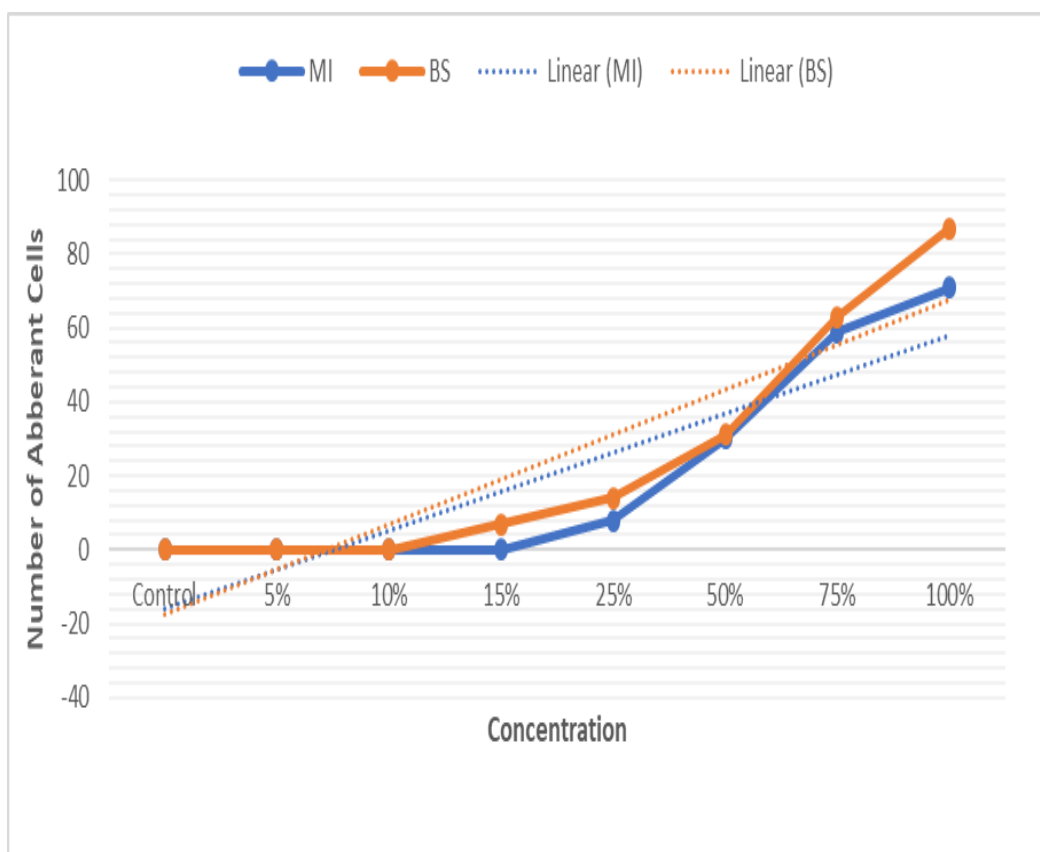
The mitotic index (MI) is a measure of the number of cells undergoing mitosis in a given cell population, reflecting the overall proliferation rate (Khakdan and Piri, 2012). The phase index, on the other hand, refers to the proportion of cells in specific phases of mitosis (prophase, metaphase, anaphase, and telophase) (Pathiratne et al., 2015). A concentration-dependent decrease in both the mitotic index and phase index in *Allium cepa* roots indicates that the plant extracts inhibit cell division, with greater concentrations leading to more pronounced effects (Pathiratne et al., 2015). Compounds in the plant extracts can induce cytotoxic and genotoxic effects, causing cell cycle arrest or cell death. This reduces the number of cells entering and progressing through mitosis (Khakdan and Piri, 2012).



**Figure-I** Compared effect of different concentrations of *Blighia sapida* and *Mangifera indica* in the root number of *Allium cepa* root



**Figure-II** Compared effect of different concentrations of *Blighia sapida* and *Mangifera indica* in the root length of *Allium cepa* root



**Figure-III:** Compared effect of leaf extract of *Mangifera indica* and *Blighia sapida* on [A] Total Aberrant Cells

**Table-I** Materials Used for the Research

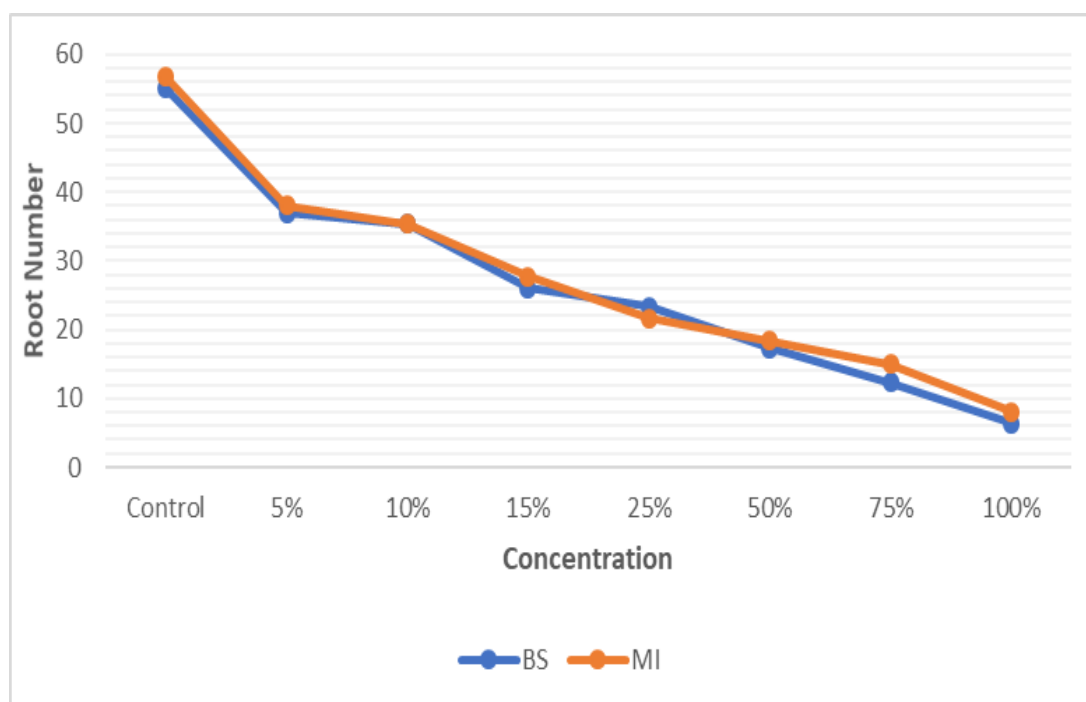
S/N	Description	Materials
	Selected Plant Materials	Dried Leaves of Mango ( <i>Mangifera indica</i> ) and Ackee Apple ( <i>Blighia sapida</i> )
	Grinding	SC-1589 Double Cup Powerful Multifunctional Blender (5000W)
	Storage	Beakers, bijou bottles, and a freezer for sample preservation
	Sieving	Cheese cloth
	Growing	Plastic disposable cups for onion bulbs
	Microscopic Evaluation	Microscopic slides, coverslips, fine forceps, sharp razor-blade, dropper, LED Light Laboratory Research microscope, and mobile device for photomicrographs
	Miscellaneous	Stopwatch for timing
	Reagents	Tap and distilled water, 0.1N Hydrochloric acid, Carnoy's solution (1:3 acetic acid and absolute ethanol) and acetocarmine (stain)

**Table-II** Antimalaria Botanicals used in Ekiti State, Nigeria

	Local and Scientific Name	Plants Part used	Frequency of Used	Perceived Efficacy	Preparation Method	Administartion Method
1	Mangoro ( <i>Mangifera indica</i> )	Leaves, Bark	High	High	Decoction of leaves and bark in boiled water	Oral consumption of decoction
2	Guava ( <i>Psidium guajava</i> )	Leaves	High	High	Infusion of leaves in hot water	Drinking infusion
3	Ishin, Ackee apple ( <i>Blighia sapida</i> )	Leaves and Seeds	High	High	Leaves and seeds crushed and mixed with water	Oral consumption of mixture
4	Cashew ( <i>Anarcadium occidentale</i> )	Leaves, Barks	High	High	Decoction of leaves and bark in boiled water	Oral consumption of decoction
5	Akintola, Siam weed ( <i>Chromolaena odorata</i> )	Leaves	Medium	Medium	Infusion of leaves in hot water	Drinking infusion
6	Agunmaniye ( <i>Gliricidia sepium</i> )	Leaves, Bark, Seeds	Medium	Medium	Crushed leaves, bark, and seeds mixed with water	Oral consumption of mixture
7	Dongoyaro ( <i>Azadiratcha indica</i> )	Leaves, Bark	High	High	Decoction of leaves and bark in boiled water	Oral consumption of decoction
8	Oruwo ( <i>Morinda lucida</i> )	Leaves, Bark	Medium	Medium	Infusion of leaves and bark in hot water	Drinking infusion
9	Lapalapa ( <i>Jastroph curcas</i> )	Leaves, Seeds	Medium	Medium	Crushed leaves, and seeds mixed with water	Oral consumption of mixture
10	Ayin ( <i>Hunteria umbellata</i> )	Seeds	Low	Low	Seeds ground into powder and mixed with water	Oral consumption of mixture

**Table-III** Effects of Different Concentrations of *Mangifera indica* on the Root Inhibition of *A. cepa* root

Concentration (%)	Root length (cm)	Number of roots	% Root Growth
0	4.10±0.05 <sup>a</sup>	56.67±1.45 <sup>a</sup>	100±0.00 <sup>a</sup>
5%	3.67±0.08 <sup>b</sup>	38.00±1.52 <sup>b</sup>	64.00±0.44 <sup>b</sup>
10%	3.70±0.05 <sup>b</sup>	35.33±0.33 <sup>b</sup>	43.05±0.43 <sup>c</sup>
15%	3.53±0.09 <sup>b</sup>	27.67±1.20 <sup>c</sup>	29.86±0.43 <sup>d</sup>
25%	2.90±0.06 <sup>c</sup>	21.67±0.88 <sup>d</sup>	16.67±0.41 <sup>e</sup>
50%	1.63±0.09 <sup>d</sup>	18.33±0.58 <sup>de</sup>	10.42±0.37 <sup>e</sup>
75%	0.73±0.09 <sup>e</sup>	15.00±0.58 <sup>e</sup>	7.63±0.34 <sup>ef</sup>
100%	0.60±0.06 <sup>e</sup>	8.00±0.88 <sup>f</sup>	4.17±0.32 <sup>f</sup>



**Figure IV:** Compared effect of leaf extract of *Mangifera indica* and *Blighia sapida* on the Percentage of Aberrations of *A. cepa* root

**Table-IV** Effects of Different Concentrations of *Blighia sapida* on the Root Inhibition of *A. cepa* root plants on the Allium root morphology.

Concentration (%)	Root length (cm)	Number of roots	%Root Growth
0	4.33 ±0.24 <sup>a</sup>	55.00 ±0.00 <sup>a</sup>	100±0.00 <sup>a</sup>
5%	3.63 ±0.21 <sup>b</sup>	37.00 ±0.21 <sup>b</sup>	64.00±0.44 <sup>b</sup>
10%	3.17 ±0.33 <sup>c</sup>	35.33 ±0.01 <sup>b</sup>	43.05±0.43 <sup>c</sup>
15%	3.07 ±0.22 <sup>c</sup>	26.00 ±0.01 <sup>c</sup>	30.86±0.43 <sup>d</sup>
25%	2.83 ±0.21 <sup>c</sup>	23.33 ±0.21 <sup>cd</sup>	16.67±0.41 <sup>e</sup>
50%	1.50 ±0.21 <sup>d</sup>	17.33 ±0.21 <sup>de</sup>	11.42±0.37 <sup>e</sup>
75%	0.93 ±0.25 <sup>e</sup>	12.33 ±0.01 <sup>ef</sup>	7.63±0.34 <sup>ef</sup>
100%	0.53 ±0.24 <sup>e</sup>	6.33 ±0.00 <sup>f</sup>	5.17±0.32 <sup>f</sup>

**Table-V** Effects of Crude Leaf Extract of *Mangifera indica* on the Mitotic Index and the Percentage Chromosomal Aberration of *A. cepa* Root

Conc. (%)	TCC	Prophase	Metaphase	Anaphase	Telophase	TD	MI	MH
0	500±0.00 <sup>a</sup>	69.33±0.21 <sup>ab</sup>	72.33±0.04 <sup>a</sup>	55.33±0.22 <sup>a</sup>	64.67±0.14 <sup>a</sup>	263.00±0.33 <sup>a</sup>	0.72±0.01 <sup>a</sup>	0.00±0.00 <sup>h</sup>
5	500±0.00 <sup>a</sup>	70.33±0.24 <sup>a</sup>	65.00±0.02 <sup>b</sup>	55.00±0.36 <sup>a</sup>	64.33±0.24 <sup>a</sup>	254.00±1.25 <sup>b</sup>	0.51±0.00 <sup>b</sup>	27.23±1.02 <sup>g</sup>
10	500±0.00 <sup>a</sup>	67.00±0.36 <sup>b</sup>	54.67±0.02 <sup>c</sup>	51.33±0.01 <sup>b</sup>	60.33±0.11 <sup>b</sup>	233.67±5.36 <sup>c</sup>	0.47±0.01 <sup>c</sup>	32.33±0.55 <sup>f</sup>
15	500±0.00 <sup>a</sup>	49.33±0.22 <sup>c</sup>	57.00±0.33 <sup>c</sup>	51.00±0.08 <sup>b</sup>	60.33±0.24 <sup>b</sup>	220.00±4.58 <sup>d</sup>	0.43±0.00 <sup>d</sup>	40.33±1.44 <sup>e</sup>
25	500±0.00 <sup>a</sup>	51.00±0.24 <sup>c</sup>	54.67±0.47 <sup>c</sup>	44.00±0.17 <sup>c</sup>	51.00±0.00 <sup>c</sup>	191.67±3.69 <sup>e</sup>	0.36±0.00 <sup>e</sup>	48.33±1.23 <sup>d</sup>
50	500±0.00 <sup>a</sup>	41.00±0.33 <sup>d</sup>	37.00±0.15 <sup>d</sup>	43.67±0.14 <sup>c</sup>	46.67±0.24 <sup>d</sup>	171.00±1.24 <sup>f</sup>	0.33±0.00 <sup>f</sup>	54.33±0.25 <sup>c</sup>
75	500±0.00 <sup>a</sup>	41.00±0.22 <sup>d</sup>	22.67±0.24 <sup>e</sup>	41.67±0.24 <sup>c</sup>	34.67±0.01 <sup>e</sup>	141.00±1.98 <sup>g</sup>	0.26±0.00 <sup>g</sup>	62.67±0.78 <sup>b</sup>
100	500±0.00 <sup>a</sup>	27.33±0.01 <sup>e</sup>	20.67±0.14 <sup>e</sup>	26.67±3.01 <sup>d</sup>	24.67±0.01 <sup>f</sup>	101.67±1.77 <sup>h</sup>	0.19±0.00 <sup>h</sup>	72.00±0.46 <sup>a</sup>



**Table-VI** Effects of Crude Leaf Extract of *Blighia sapida* on the Mitotic Index and the Percentage Chromosomal Aberration of *A. cepa* root

Conc. (%)	TCC	Prophase	Metaphase	Anaphase	Telophase	TD	MI	MH
0	500±0.00 <sup>a</sup>	71.33±0.21 <sup>a</sup>	68.33±0.04 <sup>a</sup>	76.33±0.22 <sup>a</sup>	56.67±0.14 <sup>a</sup>	359.00±0.33 <sup>a</sup>	0.72±0.01 <sup>a</sup>	0.00±0.00 <sup>h</sup>
5	500±0.00 <sup>a</sup>	71.33±0.24 <sup>a</sup>	66.00±0.02 <sup>b</sup>	70.00±0.36 <sup>ab</sup>	55.33±0.24 <sup>a</sup>	261.00±1.25 <sup>b</sup>	0.54±0.00 <sup>b</sup>	27.30±1.02 <sup>g</sup>
10	500±0.00 <sup>a</sup>	67.00±0.36 <sup>b</sup>	63.67±0.02 <sup>c</sup>	52.33±0.01 <sup>b</sup>	52.33±0.11 <sup>b</sup>	234.67±5.36 <sup>c</sup>	0.48±0.01 <sup>c</sup>	34.82±0.55 <sup>f</sup>
15	500±0.00 <sup>a</sup>	59.33±0.22 <sup>c</sup>	57.00±0.33 <sup>d</sup>	52.00±0.08 <sup>b</sup>	44.33±0.24 <sup>bc</sup>	212.00±4.58 <sup>d</sup>	0.44±0.00 <sup>d</sup>	40.96±1.44 <sup>e</sup>
25	500±0.00 <sup>a</sup>	55.00±0.24 <sup>cd</sup>	43.67±0.47 <sup>e</sup>	50.00±0.17 <sup>c</sup>	38.00±0.00 <sup>c</sup>	186.67±3.69 <sup>e</sup>	0.38±0.00 <sup>e</sup>	48.19±1.23 <sup>d</sup>
50	500±0.00 <sup>a</sup>	50.00±0.33 <sup>d</sup>	32.00±0.15 <sup>f</sup>	43.67±0.14 <sup>cd</sup>	38.67±0.24 <sup>c</sup>	163.00±1.24 <sup>f</sup>	0.33±0.00 <sup>f</sup>	54.87±0.25 <sup>c</sup>
75	500±0.00 <sup>a</sup>	42.00±0.22 <sup>ef</sup>	26.67±0.24 <sup>g</sup>	30.67±0.24 <sup>d</sup>	32.67±0.01 <sup>d</sup>	130.00±1.98 <sup>g</sup>	0.26±0.00 <sup>g</sup>	63.78±0.78 <sup>b</sup>
100	500±0.00 <sup>a</sup>	31.33±0.01 <sup>f</sup>	18.67±0.14 <sup>h</sup>	23.67±3.01 <sup>e</sup>	26.67±0.01 <sup>e</sup>	98.67±1.77 <sup>h</sup>	0.20±0.00 <sup>h</sup>	73.00±0.46 <sup>a</sup>

**Table-VII** Effects of Crude Leaf Extract of *Mangifera indica* on the Percentage Chromosomal Aberration of *A. cepa* Root

Conc (%)	MIC	GC	SC	BBC	NL	IC	MN	VA	%AB
0	0.00±0.00 <sup>d</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>d</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>e</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>d</sup>	0.00
5	0.00±0.00 <sup>d</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>d</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>e</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>d</sup>	0.00
10	0.00±0.00 <sup>d</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>d</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>e</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>d</sup>	0.00
15	0.00±0.00 <sup>d</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>d</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>e</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>d</sup>	0.00
25	0.00±0.00 <sup>d</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>d</sup>	0.00±0.00 <sup>c</sup>	<b>8.00±0.55<sup>d</sup></b>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>b</sup>	<b>8.00±0.55<sup>c</sup></b>	<b>4.20</b>
50	<b>5.00±0.00<sup>c</sup></b>	0.00±0.00 <sup>b</sup>	<b>4.00±0.24<sup>c</sup></b>	0.00±0.00 <sup>c</sup>	<b>13.12±0.00<sup>c</sup></b>	<b>8.01±0.20<sup>b</sup></b>	0.00±0.00 <sup>b</sup>	<b>30.13±0.56<sup>c</sup></b>	<b>17.54</b>
75	<b>9.07±0.06<sup>b</sup></b>	<b>10.71±0.24<sup>a</sup></b>	<b>6.02±0.01<sup>b</sup></b>	<b>6.00±0.01<sup>b</sup></b>	<b>17.00±0.00<sup>b</sup></b>	<b>11.26±0.00<sup>a</sup></b>	0.00±0.00 <sup>b</sup>	<b>60.06±0.32<sup>b</sup></b>	<b>41.55</b>
100	<b>10.00±0.34<sup>a</sup></b>	<b>11.01±0.10<sup>a</sup></b>	<b>13.21±0.31<sup>a</sup></b>	<b>7.01±0.01<sup>a</sup></b>	<b>20.00±0.00<sup>a</sup></b>	0.00±0.00 <sup>c</sup>	<b>10.00±0.46<sup>a</sup></b>	<b>71.63±1.22<sup>a</sup></b>	68.93

**Table-VIII** Effects of Crude Leaf Extract of *Blighia sapida* on the Percentage Chromosomal Aberration of *A. cepa* Root

CConc (%)	MMIC	VVG	GGC	SSC	BBC	NNL	IIC	MNL	VA	%% AB
0	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>f</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>f</sup>	0.00±0.00 <sup>d</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>f</sup>	0.99
5	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>f</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>f</sup>	0.00±0.00 <sup>d</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>f</sup>	0.00
10	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>b</sup>	0.00±0.00 <sup>f</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>f</sup>	0.00±0.00 <sup>d</sup>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>f</sup>	0.00
15	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>b</sup>	<b>4.03±0.20<sup>d</sup></b>	0.00±0.00 <sup>c</sup>	0.00±0.00 <sup>c</sup>	<b>3.00±0.00<sup>c</sup></b>	0.00±0.00 <sup>d</sup>	0.00±0.00 <sup>c</sup>	<b>7.03±0.00<sup>e</sup></b>	<b>3.30</b>
25	<b>3.10±0.00<sup>d</sup></b>	0.00±0.00 <sup>b</sup>	<b>3.20±0.40<sup>e</sup></b>	<b>3.00±0.00<sup>d</sup></b>	0.00±0.00 <sup>c</sup>	<b>5.00±0.55<sup>d</sup></b>	0.00±0.00 <sup>d</sup>	0.00±0.00 <sup>c</sup>	<b>14.00±0.55<sup>d</sup></b>	<b>7.53</b>
50	<b>5.00±0.00<sup>c</sup></b>	0.00±0.00 <sup>b</sup>	<b>8.00±0.00<sup>c</sup></b>	<b>6.00±0.24<sup>c</sup></b>	0.00±0.00 <sup>c</sup>	<b>8.12±0.00<sup>c</sup></b>	<b>4.00±0.20<sup>c</sup></b>	0.00±0.00 <sup>c</sup>	<b>31.12±0.56<sup>c</sup></b>	<b>19.01</b>
75	<b>8.06±0.06<sup>b</sup></b>	0.00±0.00 <sup>b</sup>	<b>11.00±0.00<sup>b</sup></b>	<b>9.00±0.01<sup>b</sup></b>	<b>7.00±0.01<sup>b</sup></b>	<b>12.00±0.00<sup>b</sup></b>	<b>9.06±0.00<sup>b</sup></b>	<b>7.00±0.00<sup>b</sup></b>	<b>63.12±0.32<sup>b</sup></b>	<b>48.46</b>
100	<b>12.00±0.34<sup>a</sup></b>	<b>5.02±0.10<sup>a</sup></b>	<b>13.00±0.10<sup>a</sup></b>	<b>12.00±0.31<sup>a</sup></b>	<b>9.00±0.01<sup>a</sup></b>	<b>15.00±0.00<sup>a</sup></b>	<b>12.00±0.00<sup>a</sup></b>	<b>9.00±0.46<sup>a</sup></b>	<b>87.02±1.22<sup>a</sup></b>	<b>88.77</b>

The leaf extract concentrations of *Blighia sapida* are significantly different from each other in the Mitotic index and mitotic inhibition, and decreased as the concentration increased (Leme and Marin-Morales, 2020). Many bioactive compounds disrupt microtubule dynamics, leading to faulty spindle formation, which hinders proper chromosome alignment and segregation. High concentrations of these compounds can trigger programmed cell death, further reducing the population of dividing cells. The phytochemicals identified in the extracts of *Mangifera indica*, *Blighia sapida*, include quercetin, luteolin, kaempferol, mangiferin, scopoletin, and others. These compounds are known for their bioactivity, which includes potential antiproliferative effects (Pathiratne et al., 2015).

Studies have shown that quercetin can inhibit cell proliferation by causing cell cycle arrest at various checkpoints. In *Allium cepa*, quercetin's ability to induce DNA damage and spindle apparatus disruption likely leads to a concentration-dependent decrease in MI and phase index (Sileshi et al., 2023). Rutin can interfere with microtubule assembly, causing a reduction in the number of cells progressing through mitosis. Mangiferin, known for its antimitotic properties, mangiferin can cause cell cycle arrest by interfering with DNA replication and mitotic spindle formation. This leads to a significant decrease in the MI and phase index as observed in *Allium cepa* roots (Asanga et al., 2023). This compound has been shown to cause G2/M phase arrest, which prevents cells from

entering mitosis and reduces the overall mitotic index.

Scopoletin induces cytotoxicity and genotoxicity, which can cause cell cycle arrest and apoptosis, reducing the MI and phase index in a concentration-dependent manner (Olaniyi, 2022). Persin, also known for its antiproliferative effects, persin can also lead to reduced mitotic activity by causing cell cycle arrest and apoptosis. It has been reported that Kaempferol induce mitotic arrest and apoptosis by disrupting microtubule dynamics, thereby lowering the MI and phase index in *Allium cepa* roots (Mohamad and Wenli, 2023). Multiple studies have documented similar effects of plant extracts on the mitotic index and phase index. Akinboro and Bakare (2007) reported that aqueous extracts of five medicinal plants caused a significant concentration-dependent decrease in the mitotic index of *Allium cepa* roots, attributing this to the genotoxic and cytotoxic properties of the extracts. Celik and Aslantruk (2020) found that *Inula viscosa* leaf extracts led to a marked decrease in the MI and phase index, correlating with the presence of bioactive compounds that interfere with mitosis. Khakdan and Piri (2012) observed that the aqueous root extract of *Arctium lappa* caused a concentration-dependent decrease in the mitotic index of *Allium cepa*, suggesting that the extract's compounds induced cell cycle arrest and apoptosis.

The concentration-dependent decrease in the mitotic index and phase index in *Allium cepa* roots exposed to extracts of *Mangifera indica* and *Blighia sapida*, highlights the significant antiproliferative effects of these plant compounds. The observed effects are primarily due to the genotoxic and cytotoxic properties of phytochemicals such as quercetin, mangiferin, kaempferol, and scopoletin, which interfere with cell cycle progression, spindle formation, and induce apoptosis. These findings are consistent with existing literature that documents similar inhibitory effects of plant extracts on cell division, underscoring the importance of evaluating the potential genotoxicity of these botanicals, particularly in their use as therapeutic agents.

## Conclusion

This study evaluated the cytotoxic and genotoxic effects of *Mangifera indica* and *Blighia sapida* leaf extracts on *Allium cepa* root growth and mitotic activity. The results revealed a concentration-dependent inhibition of

root length and number, with significant reductions observed at higher extract concentrations. *Mangifera indica* reduced root length from 4.2 cm (control) to 0.6 cm at 100%, while *Blighia sapida* decreased it from 4.33 cm to 0.53 cm. Similarly, the number of roots declined markedly with increasing extract concentrations. Mitotic index analysis showed a progressive decrease, with values ranging from 72% to 19% for *Mangifera indica* and 72% to 20% for *Blighia sapida*. Chromosomal aberrations, including anaphase bridges and sticky chromosomes, were more pronounced in *Blighia sapida*, indicating stronger genotoxic effects. Phytochemical analysis identified bioactive compounds such as quercetin and mangiferin, which may disrupt cell division. Statistical analysis confirmed significant differences at  $p < 0.05$ .

The findings highlight the potent cytotoxic and genotoxic effects of *Mangifera indica* and *Blighia sapida* leaf extracts, with *Blighia sapida* exhibiting a more severe impact. The concentration-dependent inhibition of root growth, mitotic activity suppression, and chromosomal aberrations suggest that these plant extracts interfere with normal cell cycle processes, likely due to their bioactive phytochemicals. The presence of quercetin and mangiferin as reported by existing literature underscores their potential to disrupt microtubule dynamics, induce apoptosis, and inhibit cell proliferation. Given these effects, caution is necessary when considering these plants for medicinal or agricultural applications. Further studies on their toxicity mechanisms and potential therapeutic thresholds are essential to ensure safe usage.

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