J. Biomol. Toxicol., 1(1), 30-38, 2025
https://doi.org/10.56717/jbt.2025.v01i01.04
eISSN: 0000-0000

Journal of Biomolecular Toxicology

Research Article

Evaluation of genotoxic and cytotoxic activities of *Pterocarpus mildbraedii* ethanol leaf extract on root meristem of *Allium cepa* bulb

Ugonma Florence Uwaeme[1*], Chinyelu Clementina Osigwe[1], Jude Efiom Okokon[1,2], Christopher Ime Akpan[2], Blessing Mfon Jacob[2] and Imoh Imeh Johnny[3]

1. Department of Pharmacology and Toxicology, Faculty of Pharmacy, Madonna University, Elele, Rivers State.
2. Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.
3. Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.

Abstract

Pterocarpus mildbraedii Harms (family Fabaceae) leaves which have been used as vegetable and medicine traditionally to treat various diseases in Nigeria were investigated for their genotoxic and cytotoxic effects using *Allium cepa* assay technique. The effect of *Pterocarpus mildbraedii* extract on the root meristem cells of *Allium cepa* was evaluated using onion bulbs exposed to different concentrations of the extract (2.5, 5 and 10 mg/mL) for macroscopic and microscopic analysis. Tap water was used as a negative control and Methotrexate (0.1 mg/mL) was used as a positive control. There was a statistically significant ($p < 0.05$) inhibition of root growth, which was concentration dependent by the extract, compared with the negative control group. All tested concentrations of the extract were observed to have cytotoxic effects on cell division in *A. cepa*. Extract-induced chromosomal aberrations and micronuclei (MNC) formation in *A.cepa* root tip cells were significant ($p < 0.05$) when compared with the control groups. The treatment by extract further induced cell death, ghost cells, cell membrane damages, and binucleated cells. These results concluded that the leaf extract of *Pterocarpus mildbraedii* exerts cytotoxic and genotoxic effects on *A. cepa*.

Article Information
Received: 12 September 2025
Revised: 21 October 2025
Accepted: 22 October 2025
Published: 30 October 2025

Academic Editor
Prof. Dr. Raffaele Capasso

Corresponding Author
Prof. Dr. Ugonma Florence Uwaeme
E-mail: uflorenceugonma@gmail.com
Tel: +234-8037094265

Keywords
Pterocarpus mildbraedii, cytotoxic, genotoxic, *Allium cepa*, apoptosis.

1. Introduction

Plants that are often used as food or in trado-medicine have the potential to cause cytotoxic and genotoxic effects due to their toxic constituents [1-5]. These plants are employed in the preparation of various herbal preparations, treatment of different diseases as well as in the preparation of soups for human consumption. However, the effects of short and long term use on various organs and systems have not yet been investigated. Information on the toxic manifestations of most of these plants used in vegetables or medicine is lacking. There are reports of potential toxic effects associated with the use of these edible plants in physiological organs and systems [1-5]. Some of them have been documented for their cytotoxic and genotoxic potential [6], and some constituents of medicinal plants have been found to be sources of mutagenic, genotoxic, clastogenic, cytotoxic, carcinogenic and teratogenic compounds [7], raising concerns about the safety of long-term use of such plants, especially as edible vegetables.

Pterocarpus mildbraedii (Fabaceae) Harms is a perennial and semi-deciduous non-climbing tree that can reach

25 m in height and has a small rounded crown, commonly called Oha in Igbo and mkpafere in Ibibio. It is distributed in West and Central Africa from Guinea to the Democratic Republic of Congo, and is also found in the Usambara mountains of Tanzania [8, 9]. The leaves of *Pterocarpus mildbraedii* are used as vegetable in Nigeria.The Igbo tribe of Eastern Nigeria and some tribes in Southern Nigeria use the leaf extracts in the treatment of headaches, pains, fever, convulsions, and respiratory disorders and as antimicrobial agents [10] Biological activities reported on*Pterocarpus mildbraedii*include anti-inflammatory [11], anti-diabetic, antioxidant [10-11, 12-14], hepatoprotective [15-19], thrombolytic [11], nephroprotective [20, 21], hypolipidemic [22] cardioprotective [23], and antimicrobial [14, 24, 25] potentials. The phytochemical screening of *Pterocarpus mildbraedii* leaves reported to have alkaloids, flavonoids, tannins, saponins and cardiac glycosides [10, 25, 26]. Phyto-constituents identified in the leaves include lupeol, oleic acid, palmitic acid, 1,2,3,4 butanetetrol or erythritol, N, N dimethyl-2-propyn-1-amine, 1,2-benzenediol, 4-hydroxypiperidine, and n-hexadecanoic acid [27]. Here, we report the genotoxic and cytotoxic activities of *P. mildbraedii* leaf extract.

2. Materials and methods

2.1. Plants collection

The fresh leaves of *P. Mildbraedii* were collected in November, 2024 from a Farmland in Uyo, Uyo LGA, Akwa Ibom State, Nigeria. The leaves were identified and authenticated as *Pterocarpus mildbraedii by* a taxonomist (Prof. Margaret Bassey) at the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. The herbarium specimens were deposited at the Faculty of Pharmacy Herbarium, University of Uyo, Nigeria. Bulbs of *A. cepa*, which were purchased at the Itam market in Itu LGA of Akwa Ibom State, Nigeria, were identified and authenticated as described above.

2.2. Extraction of the leaf of P. Mildbraedii with 95% ethanol

The plant parts (leaves) were washed in running water, air-dried on a laboratory table for 2 weeks and then milled into a fine powder using a locally fabricated grinder. The powdered leaves (1 kg) were soaked in 5 L 95% ethanol for 72 h, filtered through Whatman paper No. 1 and evaporated to dryness in a water bath at 60°C. The yield (3.16 %) from 1kg of plant material was stored in a refrigerator at -4 °C until it was used for the experiment reported in this study.

2.3. Allium cepa test

2.3.1. Root growth inhibition assay (cytotoxicity)

This was carried out according to the method of komolafe et al. [28] and Ikechukwu et al. [29]. The bulbs were processed for the study using a small sharp knife to scrape the outer dried scales and remnants of old roots without destroying the root primordial to promote the emergence of new roots. All 15 bulbs were placed separately in 50 mL beakers containing distilled water to initiate root growth. The stock solution was prepared with distilled water (200 mL) by dissolving 20 g of the ethanol extract of *P. mildbraedii* in it. Concentrations of the extract using distilled water (2.5, 5 and 10 mg/mL) were prepared from the stock solution for the test in 50 mL beakers and arranged in a series of 5 per test concentration. The *A. cepa* bulbs were transferred into beakers containing graded concentrations of extract by placing each on top of each beaker, with the root primordia downward toward the liquid and arranged in a series of 5 bulbs per test concentration. Distilled water was used as a negative control and methotrexate (0.1 mg/mL) was used as positive control. The test solutions were changed every 24 h and photomicrographs of the growing *A. cepa* roots were taken. This was continued for 72 h, after which the roots were counted per beaker in all the tested concentrations and control groups and the mean root number was calculated. Similarly, the best developed 10 roots of each onion in each group were measured with a ruler and the mean root length was calculated.

The percentage (%) growth inhibition =

$$\frac{\text{Average length of roots length of test solution}}{\text{average lenght of roots length of control}} \times 100$$

2.3.2 Evaluation of cytotoxic and genotoxic of the extracts using A. cepa

Root tips were cut at a length of 10 mm from the bulbs and fixed in 3:1 (v/v) ethanol: glacial acetic acid for 24 haccording to Ikechukwu et al. [29] before being place in sample bottles and stored in a refrigerator until use.

https://doi.org/10.56717/jbt.2025.v01i01.04

Table 1. Cytotoxicity of *P. mildbraedii*leaf extract on growing roots of *A. cepa.*

Treatment group	Concentration of extract (mg/mL)	Average root Number ± S.D	Average root length (cm) ± S.D
Negative control	Tap water	26.40±3.82	4.82±0.12
Methotrexate	0.1	2.10±0.02[a]	0.10±0.01[a]
	2.5	10.0±0.01[a]	0.52±0.03[a]
Pterocarpus mildbraedii	5.0	2.00±0.16[a]	0.26±0.04[a]
	10.0	0.00±0.00[a]	0.00±0.00[a]

Values are expressed as mean ± SEM (n=5). Significant at [a]$p < 0.05$ when compared to negative control.

2.3.3. Microscopy

Root tips were washed with distilled water, hydrolysed with 1M HCL for 5 min at room temperature, and then heated at 50 °C for 6 min in a test tube. Thereafter, the root tips were placed on microscopic glass slides on a blank background with a forceps and cut off at the terminal tips. Two drops of 2% (w/v) orcein stain were added, left to stand for 2 min for proper staining, and mixed with the rootlets by gentle tapping and stirring with a stirring spatula [29].

A coverslip was placed at 45 °C to avoid air bubbles. After that, the cells were squashed with a metal rod and two additional drops of 2% w/v orcein stain were added. Excess stain was removed with tissue paper by pressing slightly down with the thumb. Five slides were prepared for each concentration, according to Ikechukwu et al. [29]. The cover slip was sealed with a clear fingernail polish and each slide was examined under a Light Microscope at a magnification of x40. Microphotographs were taken to observe chromosomal aberrations. The number of dividing cells, non-dividing cells and cells with chromosomal aberrations in the microscope fields were counted and recorded. Mitotic index and frequency of chromosomal aberration were calculated based on the number of aberrant cells per total cells counted at each concentration of the test extract [30, 31]. The mitotic inhibition was determined using the following formula:

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

$$\text{Aberrant cells (\%)} = \frac{\text{Number of Aberrant cells}}{\text{Total number of cells}} \times 100$$

$$\text{Root growth of control (\%)} = \frac{\text{Overall mean root length of test solution}}{\text{Overall mean root length of control}} \times 100$$

The following indices were considered for the evaluation of cytotoxicity and genotoxicity: (i) the mitotic index (MI) was calculated as the ratio between the number of mitotic cells and the total number of cells scored and expressed as a percentage (ii) chromatin aberrations observed and identified under the microscope (stickiness, bridges, breaks and polar deviation) were used as endpoints for the assessment of cytogenetic effects and (iii) micronuclei (MNC) were scored in interphase cells per 500 cells.

2.4. Statistical analysis

Data obtained from this work were analysed statistically using one-way ANOVA followed by the Tukey-Kramer multiple comparison test using Instat Graph-pad 5 (prism) software, (San Diego, USA). Differences between the means were considered significant at the 5% level of significance i.e. $p \leq 0.05$.

3. Results

These results show that all tested concentrations of *Pterocarpus mildbraedii* leaf extract caused significant inhibition of roots' growth in comparison to the negative and positive control groups. The root number and root length decreased as the leaf extract concentration increased. The average root lengths in the negative and positive control (methotrexate) groups were 4.82 ± 0.12 and 0.10 ± 0.01 cm respectively. However, average root lengths in 2.5, 5.0 and 10.0 mg/mL treatment groups were observed to have decreased significantly relative to negative control; 0.52 ± 0.03, 0.26 ± 0.04 and 0.00 ± 0.00 cm respectively for *P. mildbraedii* (Table 1). The mean root lengths in the treatment groups decreased significantly ($p < 0.05$) relative to the negative control. Root growth was not observed at 10 mg of test extract. Root morphology and appearance were normal in the

https://doi.org/10.56717/jbt.2025.v01i01.04

Table 2. Dividing and total cells counted under microscopic observations and mitotic values in control and treatment concentrations.

Treatment group	Concentration of extract (mg/mL)	Total number of cells	Dividing cells	M.I (%) ± S.E
Negative control	Tap water	500	354	70.80±3.22
Methotrexate	0.1	500	14	2.80±0.10[a]
	2.5	500	20	4.00±0.02[a]
Pterocarpus mildbraedii	5.0	500	16	3.20±1.18[a]
	10.0	500	0	0.00±0.00[a]

Values are expressed as mean ± SEM (n=5). Significant at [a]$p < 0.05$ when compared to negative control.

Table 3. Chromosomal and mitotic aberrations in the root meristematic cells of *Allium cepa* after treatment of extract of *P. Mildbraedii* identified from the microscopy.

Treatment group	Concentration of extract (mg/mL)	Chromosome breaks (%) ± S.E	Stickiness (%) ± S.E	Polar deviation (%) ± S.E	Aberrant cells (%) ± S.E	MNC (%) ± S.E
Negative control	Tap water	-	0.28±0.02	0.15±0.01	2.03±0.13	-
Methotrexate	0.10	3.23±1.38[a]	34.12±2.38[a]	15.23±2.34[a]	51.12±3.42[a]	3.12±0.56[a]
	2.5	2.06±0.90[a]	16.45±1.56[a]	1.03±0.68[a]	36.69±5.42[a]	2.38±0.55[a]
Pterocarpus mildbraedii	5.0	2.54±0.55[a]	24.78±4.28[a]	6.78±0.70[a]	42.18±6.96[a]	1.15±0.57[a]
	10.0	-	-	-	-	-

Values are expressed as mean ± SEM (n=5). Significant at [a]$p < 0.05$ when compared to negative control.

negative control group, but root tips treated with 2.5 mg/mL of *P. mildbraedii*leaf extract, appeared slightly yellow and at 5 mg/mL of *P. mildbraedii* leaf extract, the root tips appeared brownish (Table 1).

3.1. Cytogenetic analysis

Mitotic index is one of the parameters of cytogenetic analysis. Table 2 shows the effects of the *P. mildbraedii* leaf extract on the cytogenetic parameters of *A. cepa* roots. Cytogenetic analysis showed that the leaf extract caused a concentration-dependent and significant ($p<0.05$) decrease in the mitotic index relative to that of the negative control group. The leaf extract of *P. mildbraedii*at 2.5, 5.0 and 10 mg/mL had mitotic index of 4.00 ± 0.02, 3.20 ± 1.18 and 0.00 ± 0.00 as compared to 70.80 ± 3.22 recorded in the negative control group (Table 2).

Cytogenetic alterations caused by the extracts are shown in Table 3. Chromosomal and cytological alterations were observed in the negative control, methotrexate and *P. Mildbraedii* leaf extract-treated groups are shown in Table 3. Analysis of chromosome aberrations observed in the study showed that fragmented chromosomes, apoptotic bodies, polar deviation and binucleated cells were detected in the different concentration treatments (Table 3) (Fig. 1A and 1B). This difference was significant ($p < 0.05$) compared to the negative control group. Fragmentation of chromosome was observed in the leaf extract at the concentration of 5 mg/mL (Table 3, Fig. 1A and 1B). Laggard and membrane damaged cells were observed at the two concentrations (2.5 and 5 mg/mL) (Fig. 1C) in the extract-treated groups. Sticky and fragmented chromosomes, apoptotic bodies, membrane and nuclear damaged cells were also observed (Table 3 and Figs. 1D to 1F). These abnormalities increased with increasing extract concentrations. A concentration-dependent and statistically significant ($p < 0.05$) increase in the total aberrant cells (aberrant cells include bridge, laggard and stickiness) relative to the negative control was observed (Table 3). However, the highest frequencies of aberrant cells were observed in the methotrexate-treated group (positive control) (Table 3). Micronuclei induction in the root tip meristem cells of *A. cepa* was observed but was not concentration-dependent, as the groups treated with methotrexate and 2.5 mg/mL of *Pterocarpus mildbraedii* had a higher number of cells with micronuclei in the experiment compared to the

https://doi.org/10.56717/jbt.2025.v01i01.04

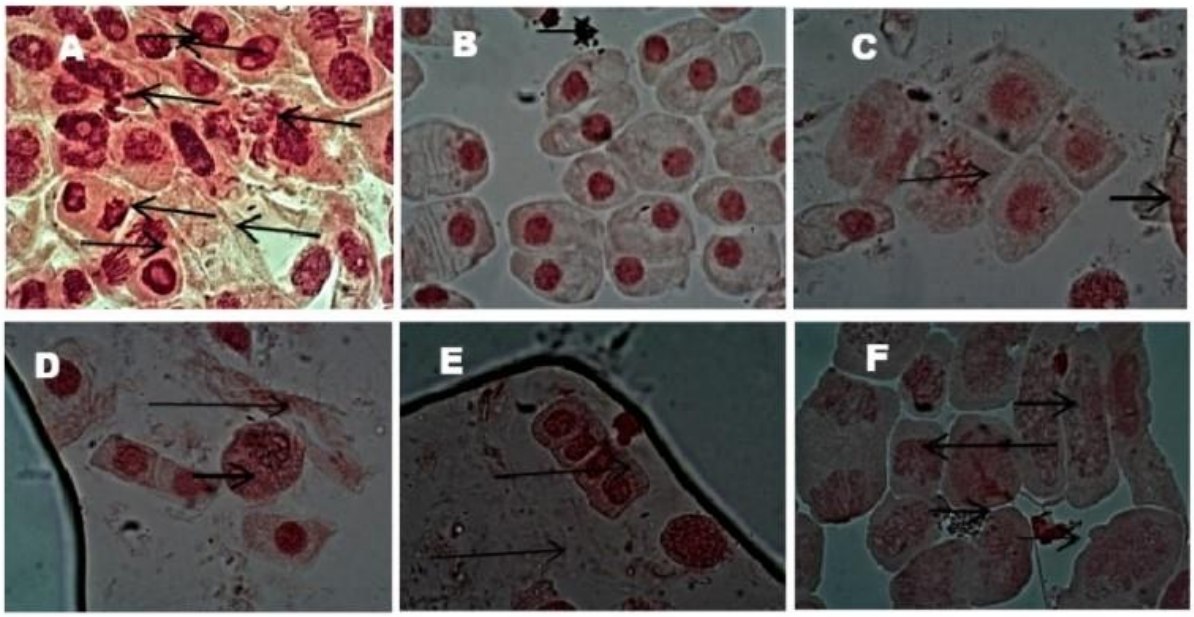

Figure 1. Photomicrograph showing the mitotic and chromosomal aberrations of *Allium cepa* root meristem cells after *Pterocarpus mildbraedii* leaf extract treatments under light microscope X40 magnification. Arrows indicate (A) apoptotic bodies,fragmented chromosome, polar deviation, binucleated cell (B) fragmented chromosomes (C) laggard, cell wall damage (D) sticky chromosome (E) apoptotic bodies, sticky chromosome (F) cell wall and nuclear damage, fragmented and sticky chromosomes.

negative control, which was statistically significant ($p < 0.05$) (Fig. 1D).

4. Discussion

In this study, the toxic effects of *Pterocarpus mildbraedii* leaf extracts were assessed by evaluating the growth and morphology of *A. Cepa* roots. In the root growth inhibition assay, root growth was inhibited by various concentrations of the extract, as observed in this study and these were statistically significant compared to the control group. Furthermore, the extract caused light yellow, light brown and brownish root colouration. The root growth inhibition assay is an established method for detecting and quantifying of the cytotoxic and genotoxic potential of various compounds, including phytoconstituents of medicinal plants [32]. The root growth inhibition observed in this study may be due to the presence of growth inhibitory allelochemicals in the extract and might also be due to the inhibition of cell division, which is active at the tip of the root of *A. cepa* exposed to different concentrations of the extract. This observation was in agreement with the study carried out by [33] on the growth inhibitory effects of *Zea mays* husk and *Sacharum officinarum* leaf on *A. cepa* root.

Cyto- and genotoxicity were determined by assessing cytological parameters such as the mitotic index and number of chromosome abnormalities, including chromosome breaks, stickiness, and polar deviations. The cells of *A. cepa* root tips after treatment with the extracts showed a decreased mitotic index with increasing concentrations. The mitotic index (MI) of *A. cepa* meristematic cells treated with methotrexate (2.80%) was significantly lower than that of control. Significant inhibition was observed in the onion roots treated with *Pterocarpus mildbraedii* leaf extract (4.00%, 3.20% and 0.00%) compared to the negative control (Table 2).

The inhibition of root growth was found to increase with decreased Mitotic Index. The reduction of mitotic index below 22% in comparison to the negative control can have a lethal impact on the organism [34], while a decrease below 50% usually has sub-lethal effects [35] and is called the cytotoxic limit value [36]. The mitotic index measures the proportion of cells in the M-phase of the cell cycle and its inhibition can be interpreted as cellular death or a delay in cell proliferation kinetics [37]. The reduction in mitotic activity could be due to the inhibition of DNA synthesis or blocking in the G2 phase of the cell cycle,

https://doi.org/10.56717/jbt.2025.v01i01.04

preventing the cell from entering mitosis [38]. The mito-depressive effects of some herbal extracts, including their ability to block the synthesis of DNA and nucleus protein, were reported earlier [39, 40]. Several other herbal extracts have been reported to inhibit mitosis [29, 41, 42]. The decreased mitotic index in *A. cepa* roots treated with *Pterocarpus mildbraedii*leaf extract was probably due to either disturbance in the cell cycle or chromatin dysfunction induced by extract–DNA interactions. The results herein suggest that the tested extract concentrations have inhibitory, mito-depressive effects on root growth and cell division of *A. cepa* via prevention of DNA synthesis and a reduction in the number of the dividing cells in roots produced by the cytotoxic effects of compounds found in the extract. The observation of sticky metaphase supports the hypothesis of toxic effects of the extract. Metaphases with sticky chromosomes, lose their normal appearance, and appear with a sticky "surface," causing chromosome agglomeration [43]. Stickiness has been attributed to the effect of pollutants and chemical compounds on the physicochemical properties of DNA, proteins or both, on the formation of complexes with phosphate groups in DNA, DNA condensation or on the formation of inter- and intra-chromatid cross links [44, 45]. Chromosomal aberrations (CA) are changes in chromosome structure resulting from a break or exchange of chromosomal material. Most of the CA observed in cells are lethal, but there are many related aberrations that are viable and that can cause genetic effects, either somatic or inherited [46]. The presence of chromosome fragments is an indication of chromosome breaks, and can be a consequence of anaphase/telophase bridges [47]. Fragment was not observed in this study in all the extract concentrations- treated groups. The extract was found to interfere with the cell cycle and affect chromatin organization or DNA replication. The frequencies of total chromosomal aberrations increased significantly following exposure to the extract indicating clastogenic activity (Table 3). The extract significantly induced MNC formation in *A. cepa* root cells at 2.5-5 mg/mL concentrations. The frequency of MNC increased in the groups treated with 2.5 mg/mL of the leaf extract. However, there was no root growth at the highest concentration of the extract (10 mg/mL), perhaps due to the high cytotoxicity. The frequency of cells with micronuclei is a good indicator of the cytogenetic effects of the tested chemicals. Micronuclei (MNC) often results from the accentric fragments or lagging chromosomes that fail to incorporate into the daughter nuclei during telophase of the mitotic cells and can cause cellular death due to the deletion of primary genes [48, 49]. Previous studies have suggested MNC-induced effects of various plant extracts, such as *Hippocratea africana* [41], *Setaria megaphylla* [42], *Heinsia crinata, Lasianthera africana, Justicia insularis* [29], *Solanum anomalum* fruit [50] and *Croton zambesicus* [51].

In this study, cells with membrane damage were observed in all the treated groups. These findings suggested that the extract might have cytotoxic effects at specific concentrations including damaging membranes. Multinucleated and binucleated cells were observed in extract-treated groups. This is due to the prevention of cytokinesis or cell plate formation. Microtubules have been implicated in cell plate formation by the extract, resulting in inhibition of cytokinesis. Ghost cells are dead cells in which the outline remains visible, but whose nucleus and cytoplasmic structures are not stainable [52]. Some ghost cells were observed at various frequencies in this study especially in the 10 mg/mL treated group (Fig. 1). This could have resulted from the activities of the phytoconstituents of the extract leading to nuclear damage and the prevention of cytoplasmic structures. In addition, the extract also induced DNA damage, cell death and/or apoptosis at various frequencies in this study. Cell death is a basic biological process of living organisms. The cell death is induced by high concentrations of toxins, stress, heavy metals, chemicals and other factors. Phyto-constituents identified in the leaves include lupeol, oleic acid, palmitic acid, 1,2,3,4 butanetetrol or erythritol, N, N dimethyl-2-propyn-1- amine, 1,2-benzenediol, 4-hydroxypiperidine, and n-hexadecanoic acid [27]. The phytoconstituents of this extract may have been responsible for the observed effects in this study.

However, Considering the sensitivity of the *A.cepa* test and related genotoxic tests, one can determine the influence of toxic substances on a healthy organism and show excellent correlation with similar tests, like

https://doi.org/10.56717/jbt.2025.v01i01.04

Amnes test on animals and other vertebrates. Although these results can be extrapolated to humans, there may be in vivo variations. Further work on other aspects of toxicology (toxic effects of plant extracts on organ functions) of the extract should be recommended. Activity guided assays and phytochemical profiling for predictive toxicology are recommended for further work.

5. Conclusions

The results of this study show that the leaf extract of *Pterocarpus mildbraedii* can induce cytogenetic alterations and cell death in the root tips of *A. cepa*, suggesting the cytotoxic and genotoxic activities of the extract. Therefore, the proper use of these plants in ethnomedicine is recommended and high doses should be avoided as they can cause cytotoxic and/or genotoxic effects.

Disclaimer (artificial intelligence)

Author(s) hereby state that no generative AI tools such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators were utilized in the preparation or editing of this manuscript.

Authors' contributions

Conceptualization, J.E.O., C.C.O.; methodology, J.E.O.; software, U.F.U.; validation, C.C.O., J.E.O.; formal analysis, C.C.O., J.E.O., U.F.U.; investigation, K.E.; resources, C.C.O., U.F.U., J.E.O, K.E.; data curation, K.E.; writing – original draft preparation, J.E.O.; writing – review & editing, C.C.O., U.F.U.; visualization, KE., U.F.U., C.C.O.; supervision, J.E.O., C.C.O., U.F.U.; project administration, J.E.O.

Acknowledgements

The authors are grateful to the Madonna University, Elele campus and University of Uyo managements for providing enabling environment for the completion of this work.

Funding

This research received no specific grant from any funding agency (the public, commercial, or not-for-profit sectors).

Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

Authors have declared that no competing interests exist.

References

1. Okokon, J.E.; Udobang, J.A.; Uwaeme, U.F.; Osigwe, C.C.; Nyong, M.E. Toxicological evaluation of chronic administration of *Zea mays* husk extract on female rats. J. Complement. Altr. Med. Res. 2025a, 28(8), 84-97. https://doi.org/10.9734/jocamr/2025/v26i8693
2. Okokon, J.E.; Udobang, J.A.; Osigwe, C.C.; Uwaeme, U.F.; Ise, U.P.; Edem, U.A. Histopathological study of subacute administration of *Saccharum officinarum* leaf extract on some organs of rat. J. Complement. Altr. Med. Res. 2025b, 26(1), 12-20. https://doi.org/10.9734/jocamr/2025/v26i1611
3. Okokon, J.E.; Etuk, I.C.; Osigwe, C.C.; Uwaeme, U.; Udobang, J.A.; Ise, U.P. Effect of ninety-day repeated adiministration of leaf extract of *Solanum anomalum* on rats. J. Complement. Altr. Med. Res. 2025c, 26(1), 59 -78. https://doi.org/10.9734/jocamr/2025/v26i1614
4. Edem, U.; Okokon, J.E. Subacute toxicity study of *Saccharum officinarum* (sugarcane). Biol. Med. Nat. Prod. Chem. 2025. 14(1), 91-100. https://doi.org/10.14421/biomedich.2025.141.91-100
5. Andrew, U.A.; Okokon, J.E., Uwaeme, U.F.; Osigwe, C.C.; Ise, U.P. Subacute toxicity study of stem extract of *Telfairia occidentalis* in rodents. Asian Journal of Biochem. Gen. Mol. Med. 2025, 17(2), 19-35. https://doi.org/10.9734/ajbgmb/2025/v17i2440
6. Ikechukwu, E.; Agu, P.; Olumuji, H.; Anaboso, M.O.; Ebong, N.O.; Okokon, J.E. Evaluation of genotoxic and cytotoxic activities of three vegetables (*Heinsia Crinata, Justicia insularis a*nd *Lasianthera africana*) using *Allium cepa* test. Asian J. Biochem. Gen. Mol. Biol. 2024, 16(4), 10-20. https://doi.org/10.9734/ajbgmb/2024/v16i4367
7. Chandra, S.J.; Sandhya, S.; Vinod, K.R.; David, B.; Sudhaka, K.; Chaitanya, R. Plant toxins useful and harmful effects. J. Drg. Med. 2012, 4(1), 79-90. https://doi.org/10.3390/toxins17060270
8. Bosch, C.H. *Pterocarpus mildraedii* Harms. Plant Res. Trop. Afr. 2004, 3, 78-80. https://doi.org/10.16/j,plantsci.2003.09.020
9. Grubben, G.J.H., Denton, O.A. Vegetables. PROTA Foundation, Wageningen, Netherlands. PROTA Foundation, Wageningen. Plant Res. Trop. Afr. 2004. https://doi.org/10.1017/s0021859605245240
10. Usunomena, U.; Chinwe, I.V. Phytochemical screening,

mineral composition and in vitro antioxidant activities of *Pterocarpus mildbraedii* leaves. Int. J. Sci. World. 2016, 4(1), 23-26. https://doi.org/10.14419/ijsw.v4i1.6066

11. Fajobi, A.O.; Emma-Okon, B.O.; Oyedapo, O.O. In vitro antioxidant, anti-inflammatory and thrombolytic properties of leaf extract of *Pterocarpus mildbraedii* harms. Ife J. Sci. 2020a, 22(3), 065-074. https://doi.org/10.4314/ijs.v22i3.5
12. Omale, J.; Okafor, N.O.; Omede, A. Polyphenol content cytotoxicity and antioxidant activities of some selected Nigerian vegetable food. Int. J. Chem. Sci. 2008. 6(4), 1714-1725.
13. Ifesan, B.O.T.; Egbewole O.O.; Ifesan, B.T. Effect of fermentation and nutritional composition of selected commonly consumed green leafy vegetables in Nigeria. Int. J. Appl. Sci. Biotechn 2014, 2(3), 291-297. https://doi.org/10.3126/ijasbt.v2i3.11003
14. Abare-Jen, L.; Mensah, J.K.; Boroqouye, S.L. Evaluation of the phytochemistry, antimicrobial and antioxidant efficacy of the stem bark extracts of *Pterocarpus mildbraedii.* IOSR J. Appl. Chem. 2020, 13(5), 15-20. https://doi.org/10.46602/jcsn.v46i4.643
15. Otuechere, C.A.; Abarikwu, S.O.; Rufai, M.A.; Ohiozoje, A.E.; Martins, E.; Farombi, E.O. Protective effects of vitamin C against propanil-induced hepatotoxicity in Wistar rats. Asian Pacific J. Trop. Dis. 2012, 2, 212–217. https://doi.org/10.1016/s2222-1808(12)60154-6
16. Adegbite, A.V.; Ezekwesili, C.N.; Okani, O. Effects of ethanol and aqueous leaf extracts of *Pterocarpus mildbraedii* on the liver function and CD4 cells of the immune system in albino rats. Bioscientist J. 2015, 3(1), 79-87.
17. Hamzah, R.U.; Jigam, A.A.; Makun, H.A.; Egwim, E.C.; Muhammad, H.L.; Busari, M.B.; Ibikunle, G.F.; Abubakar-Akanbi, S.K. Effect of partially purified sub-fractions of *Pterocarpus mildbraedii* extracts on carbon tetrachloride intoxicated rats. Integr. Med. Res. 2018, 7(2), 149–158. https://doi.org/10.1016/j.imr.2018.01.004
18. Otuechere, A.C.; Farombi, E.O. *Pterocarpus mildbraedii* (Harms) extract resolves propanil-induced hepatic injury via repression of inflammatory stress responses in Wistar rats. J. Food Biochem. 2020, 44(12), e13506. https://doi.org/10.1111/jfbc.13506
19. Akinwunmi, K.F.; Amadi, C.V.; Awodiran, M.O. The effect of aqueous leaf extracts of *Pterocarpus mildbraedii* on crude oil-induced toxicity in Albino rats. J. Herbs, Spices Med. Plants 2021, 27(4), 365-376. https://doi.org/10.1080/10496475.2021.1934215
20. Ezekwesili, C.N.; Adegbite, A.V.; Okani, O.C. Effects of leaf extracts of *Pterocarpus mildbraedi*i on renal and heart functions of rats. Ani. Res. Int. 2016, 13(2), 2446-2453. https://doi.org/10.5897/ajbr2017.0930
21. Urom, E.; Usin, G.S. Nephroprotective effect of *Pterocarpus mildbraedii* leaf extract in paracetamol-induced toxicity in Wistar rats: A histopathological investigation.*GSC* Biol. Pharm Sci. 2023, 24(01), 233–239. https://doi.org/10.30574/gscbps.2023.24.1.0101
22. Adegbite, A.V.; Ezekwesili, C.N. Effects of ethanol and aqueous leaf extracts of *Pterocarpus mildbraedii* on hematology parameters and cholesterol in albino rats. Afr. J. Biochem. Res. 2017, 11(6), 28-33. https://doi.org/10.5897/ajbr2017.0930
23. Fajobi, A.; Emma-Okon, B.O.; Oyedapo, O.O.; Dare, C.A.; Akinlalu, A.O.; Komolafe, I.J.; Ogunsusi, M. Cardioprotective activities of *Pterocarpus mildbraedii* leaves on isoproterenol-induced myocardial infarction in rats. Eur. J. Mednl Plants. 2020b, 31(10), 20-37. https://doi.org/10.9734/ejmp/2020/v31i1030278
24. Amadi, E.S.; Oguoma, O.I.; Ibekwe, V.I.; Abanobi, S.E.; Chikwendu, C.I.; Egbadon, O.E. Prevalence and the effect of plant extracts on community associated methicillin resistant Staphylococcus aureus in Owerri, Imo State, Nigeria. J. Res. Biol. 2013, 3(4), 967-976.
25. Anarado, C.E.; Anarado, C.J.O.; Okechukwu, E.E.; Chukwubueze, F.M.; Kenechukwu, G.E. Comparative phytochemical and antimicrobial analyses of leaves of *Pterocarpus mildbraedii* Harms and *Xylopia aethiopica* (Dual) A. Rich. South Asian Res. J. Nat. Prod. 2021, 4(3), 157-166.
26. Akinyeye, R.O.; Oluwadunsi, A. Omoyeni, A. Proximate, mineral, anti-nutrients, phyto-chemical screening and amino acid compositions of the leaves of *Pterocarpus mildbraedi* harms. Electron. J. Environ. Agric. Food Chem. 2010, (94),1323-1333.
27. Uchegbu, R.I.; Iwuoha, G.U.; Elenwoke, U.E.; Ibe, C.O.; Amanze, K.O. Identification of phytochemicals present in the leaves of *Pterocarpus mildbraedii* (Harms) by GC/MS analysis. IOSR J. Appl Chem. 2015, 8(7), 06-10.
28. Komolafe, I.J.; Dare, C.A.; Ogunsusi, M.; Akinlalu, A.O.; Fakayode, E.A.; Oziegbe, M.; Oyedapo, O.O. Cytotoxic and genotoxic potentials of the extracts and fraction of the leaf of Datura metel (Lnn.). Nig. J. Biochem. Mol. Biol. 2022, 37(1), 32-40.
29. Uwaeme, U.F.; Okokon, J.E.; Osigwe, C.C.; Johnny, I.I.; Umoh, J.; Udo, I.J. Evaluation of genotoxic and cytotoxic activities of the ethanol extract of *Alchonea laxiflora* on root meristem of *Allium cepa* bulb. J. Pharm. Biores. *2025*. 22(3), 177-184. https://dx.doi.org/10.4314/jpb.v22i3.4
30. Bakare, A.A.; Mosuro, A.A.; Osibanjo, O. Effect of simulated leachate on chromosomes and mitosis in roots of *Allium cepa* L. J. Environ. Biol. 2000. 21, 263–271.
31. Magnus, S.P.; Anagboso, M.O.; Johnny, I.I.; Ise, U.P.; Okokon, J.E. Evaluation of genotoxic and cytotoxic

activities of leaf and seed extracts of *Telfairia occidentalis.* J. Complement. Altr. Med. Res. 2024. 25(3), 7-16. https://doi.org/10.9734/jocamr/2024/v25i3521

32. Dragoeva, A.P.; Koleva, V.P.; Nanova, Z.D.; Georgiev, B.P. Allelopathic effects of *Adonis vernalis* l.: root growth inhibition and cytogenetic alterations. J. Agric. Chem. Environ. 2015, 4, 48-55. http://dx.doi.org/:10.4236/jacen.2015.42005
33. Akpan, EE.; Johnny, I.; Anagboso, M.O.; Ebong, O.O.; Okokon, J.E. Genotoxic and cytotoxic potentials of cornhusk extracts of *Zea mays* and leaf extract of *Saccharum officinarum.* Biol. Med. Nat. Prod. Chem. 2024, 13, 531-540. https://doi: 10.14421/biomedich.2024.132.485-494
34. Antonsie-wiez, D. Analysis of the cell cycle in the root of *Allium cepa* under the influence of Leda krin. Fol. Histochem. Cytobiol. 1990. 26, 79–96. https://journals.viamedica.pl/folia_histochemica_et_cytobiologica
35. Panda B.B.; Sahu, U.K. Induction of abnormal spindle function and cytokinesis inhibition in mitotic cells of *Alliumcepa* by the organophosphorus insecticide fensulfothion. Cytob. 1985, 42(1), 67-168), 147–155.
36. Sharma, C.B.S.R. Plant meristems as monitors of genetic toxicity of environmental chemicals. Current Sci. 1983, 52, 1000–1002.
37. Rojas, E.; Herrera, L.A.; Sordo, M. Mitotic index and cell proliferation kinetics for identification of antineoplastic activity. Anti-Cancer Drugs, 1993, 4(6), 637–640. https://doi.org/10.1097/00001813-199312000-00005
38. Sudhakar, R.; Ninge, G.K.N.; Venu, G. Mitotic abnormalities induced by silk dyeing industry effluents in the cells of *Allium cepa*. Cytol. 2001, 66(3), 235–239. https://doi.org/10.1508/cytologia.66.235
39. Mercykutty, V.C.; Stephen, J. Adriamycin induced genetic toxicity as demonstrated by *Allium cepa* test. Cytologia. 1980, 45(4), 769–777. https://doi.org/10.1508/cytologia.45.769
40. Schulze, E.; Kirschner, M. Microtubule dynamics in interphase cells. J. Cell Biol. 1986, 102(3), 1020–1031, https://doi.org/10.1083/jcb.102.3.1020
41. Johnny, I.I.; Okokon, J.E.; Ochigbo, E.B.; Udo, I.J.; Adefabi, A.M. Genotoxic and cytotoxicity potentials of *Hippocratea africana.* Asian J. Biochem. Gen. Mol. Biol. 2023, 15(2), 38-45. https://doi.org/10.9734/ajbgmb/2023/v15i2331
42. Okokon, J.E.; Ochigbo, E.B.; Johnny, I.I.; Anagboso, M.O.; Ebong, N.O. Genotoxic and cytotoxicity activities of *Setaria megaphylla.* Asian J. Biochem. Gen. Mol. Biol. 2023, 15(3), 56-64. https://doi.org/10.9734/ajbgmb/2023/v15i3339
43. Babich, H.; Segall, M.A.; Fox, K.D. "The Allium test—a simple, eukaryote genotoxicity assay," Amer. Biol.Teacher. 1997, 59(9), 580–583. https://doi.org/10.2307/4450386
44. G¨om¨urgen, A.N. "Cytological effect of the potassium metabisulphite and potassium nitrate food preservative on root tips of *Allium cepa* L.," Cytol. 2005, 70(2), 119–128. https://doi.org/10.1508/cytologia.70.119
45. T¨urkoglu, S. Genotoxicity of five food preservatives tested on root tips of *Allium cepa* L.," Mutan Res./Gen. Toxicol. Environ. Mutagen. 2007, 626(1-2), 4–14. https://doi.org/10.1016/j.mrgentox.2006.07.006
46. Swierenga, S.H.H.; Heddle, J.A.; Sigal, E.A. Recommended protocols based on a survey of current practice in genotoxicity testing laboratories, IV. Chromosome aberration and sister-chromatid exchange in Chinese hamster ovary, V79 Chinese hamster lung and human lymphocyte cultures," Mutatn Res. 1991, 246(2), 301–322. https://doi.org/10.1016/0027-5107(91)90050-x
47. Sharma A.; Sen, S. Chromosome Bot. 2002. Science, Enfield, NH, USA.
48. Albertini, R.J.; Anderson, D.; Douglas, G.R. IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans," Mutatn Res. 2000, 463(2), 111–172. https://doi.org/10.1016/s1383-5742(00)00049-1
49. Krishna, G.; Hayashi, M. In vivo rodent micronucleus assay: protocol, conduct and data interpretation," Mutatn Res. 2000, 455(1-2), 155–166. https://doi.org/10.1016/s0027-5107(00)00117-2
50. Okopide, E.; Johnny, I.I.; Olamide, F.; Okokon, J.E. GC-MS analysis, in vitro antioxidant, genotoxic and cytotoxic activities of fruit extract of *Solanum anomalum.* Sumerianz J. Biotech. 2024, 7(4), 76-88. https://doi.org/10.47752/sjb.74.76.88
51. Osigwe, C.C.; Okokon, J.E.; Wilfred, U.; Uwaeme, U.F.; Johnny, I.I. Evaluation of cytotoxic and genotoxic activities of *Croton zambesicus* Muell Arg. leaf extract in rats. Trend. Nat. Prod. Res. 2025, 6(1), 1-9. https://doi.org/10.61594/tnpr.v6i1.2025.116
52. As, T.A.; kin, C.E.; Aslantu¨rk, O.S. Investigation of cytotoxic and genotoxic effects of *Ecballium elaterium* juice based on *Allium* test," Meth. Fnd. Exp. Clin. Pharm. 2009, 31(9), 591–596. https://doi.org/10.1358/mf.2009.31.9.1434629