



Cytotoxic effects of aqueous extract of *Ocimum gratissimum* using *Allium cepa* assay

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ABSTRACT

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The *Allium cepa* assay serves as a significant *in vivo* model for evaluating the cytotoxic and genotoxic potential of various substances. The increasing use of herbal remedies has raised concerns over their potential cytogenotoxic effects. This study evaluated the cytotoxic impacts of aqueous leaf extract of *Ocimum gratissimum* using the *Allium cepa* assay. Onion bulbs were exposed to different concentrations (0%, 20%, 40%, 60%, 80%, and 100%) of the extract for 10 days. The 0% treatment (containing distilled water) serves as the control. Chromosome spreads were prepared using standard protocols. Root length, mitotic index (MI), and chromosomal aberrations (CAs) were assessed. Results showed a concentration-dependent reduction in root growth and mitotic activity. The mitotic index significantly decreased with increasing extract concentration, while chromosomal abnormalities, including stickiness, bridges, and laggards, were observed. Although lower concentrations (20-40%) had less effect on the cells, the treatments still showed a significant cytotoxic effect ($p < 0.05$) compared to the control. The higher the concentration, the greater the clastogenic changes. This study demonstrated that *O. gratissimum* has potential cytotoxic and genotoxic effects at high concentrations, warranting its cautious use in traditional medicine.

1.0 Introduction

Medicinal herbs have been used in folk medicine for millennia, and their scientific evaluation has grown substantially in recent years. However, like conventional drugs, they can cause adverse effects or interact with medications, and there are limited data on their safety profiles [1, 2]. Although traditional use suggests low toxicity, green plants are known to contain both antimutagenic and toxic agents, including cytotoxic

and genotoxic compounds [3]. Recent investigations have demonstrated that many edible and medicinal plants exhibit mutagenic, cytotoxic, and genotoxic effects in both *in vitro* and *in vivo* systems [3-6]. These findings raise concerns about potential harm from long-term use, as many plants contain mutagenic or carcinogenic substances linked to increased tumour incidence in certain populations [7].

Cytotoxicity refers to the harmful effects of chemical agents on living cells, which disrupt

cellular integrity and potentially cause irreversible damage. When such agents also induce genetic changes, such as mutations, they are deemed genotoxic; a condition capable of producing severe or fatal impacts, including heritable DNA alterations in both somatic and germ cells [6, 8]. Monitoring chromosomal behaviour in mitosis or meiosis serves as a reliable method for assessing mutagenic potential [8]. Thus, cytotoxicity assays are pivotal in evaluating how chemicals, drugs, and natural extracts affect cellular structures and functions. These tests measure impacts such as alterations in membrane integrity, inhibited protein production, and receptor binding interference, through endpoints like membrane permeability, ATP levels, enzyme activity, and nucleotide uptake [8]. Consequently, there is a pressing need for fast, accurate, and cost-effective assays capable of assessing toxicity in whole organisms or cells, both *in vivo* and *in vitro*.

The *Allium cepa* assay, utilising onion root tips, is a recognised *in vivo* model for cytotoxicity and genotoxicity screening. This assay is user-friendly and economical, requiring no exogenous metabolic activation. It evaluates root growth inhibition, mitotic index, chromosomal aberrations, and nuclear anomalies to measure toxicity; studies have validated its relevance [6, 8]. It has been demonstrated that hazardous substances exposure leads to significant mitotic inhibition, chromosomal bridges, fragments, stickiness, binucleated cells, and micronuclei formation in *Allium cepa* roots. Similarly, Parvan *et al.* [9] confirmed the assay's sensitivity to environmental pollutants, herbicides, and anticancer drugs. These findings underscore the assay's strong correlation with effects observed in mammalian cells, establishing it as a valuable preliminary tool for cytogenotoxic evaluation.

Ocimum gratissimum L., commonly known as African basil or scent leaf, is a tropical plant widely used in traditional medicine across Africa, Asia, and South America. It belongs to the Lamiaceae family and is valued for its rich aromatic oils and diverse pharmacological properties. The leaves contain an array of phytochemicals, including eugenol, thymol, ocimene, flavonoids, tannins, saponins, and phenolic compounds, which are responsible for their antimicrobial, anti-inflammatory, antidiarrheal, antioxidant, and even antidiabetic effects [10]. Despite these beneficial effects, *O. gratissimum* can exhibit toxicity, especially when consumed in high doses or over extended periods [11, 12]. Studies using genotoxicity models, such as the micronucleus assay or the *Allium cepa* assay, have demonstrated that high doses can cause damage to genetic material, including the formation of micronuclei, chromosome fragmentation, and spindle disturbances. These effects suggest that some bioactive compounds may interfere with DNA

replication or repair mechanisms when present in excessive quantities [13, 14], suggesting that while the plant may be safe at low doses, its misuse or overuse could pose health risks. Some animal studies have suggested that high doses may impair reproductive function, including reduced sperm count and motility, although these findings remain limited and context-dependent [15]. Consequently, the current study aimed to evaluate the cytotoxic potential of aqueous leaf extract of *O. gratissimum* using the *Allium cepa* root meristem assay. The assessment focused on root growth inhibition, mitotic index, and chromosomal aberrations associated with the use of aqueous leaf extract to determine the extract's safety.

2.0 Materials and Methods

2.1 Plant Materials

Fresh leaves of *O. gratissimum* were harvested from a cultivated garden in Ilorin, Kwara State, Nigeria whilst healthy *Allium cepa* bulbs (2-3 cm in diameter) were obtained from a local market in Ilorin, Nigeria and assigned (UILH/001/1319/2025) and (UILH/002/1332/2025) respectively. Voucher samples were deposited at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria.

2.2 Preparation of Plant Materials and Treatments

The leaves of *O. gratissimum* were thoroughly rinsed, air-dried for two weeks at room temperature, and pulverised into fine powder using a mechanical grinder (Binatone, Japan). Fifty grams (50 g) of powdered leaves were heated in 500 mL of distilled water at 45 °C for 90 min. The mixture was filtered using Whatman No.1 filter paper and stored at 4 °C. Test concentrations of 20%, 40%, 60%, 80%, and 100% were prepared from the stock solution. Double-distilled deionised water was used as the negative control (0%).

Healthy onion bulbs (2-3 cm diameter) were obtained from a local market in Ilorin, Nigeria. After drying in the sun for five days and cleaning, healthy bulbs of approximately equal size were selected for the experiment. The bulbs were prepared by removing the dry, scaly brown covering leaves and the old roots attached to the basal stem, leaving the primordial root ring intact. The bulbs were placed in 50 mL of the prepared concentrations in conical tubes, with the basal plate submerged. Control bulbs were placed in double-distilled deionised water only. The experiment was set up in three replicates per treatment and maintained at room temperature in a dark cupboard for 10 days. The number of roots and the lengths counted/ measured at the end of exposure.

2.3 Root Harvest and Slide Preparation

When the roots of the *Allium cepa* had grown to a length of approximately 3 cm, the root tips were excised using sterilised scissors. The tips were then collected and labelled in specimen tubes according to the treatments. The pretreatment of roots and slide preparation followed the methods described by Asita and Matebesi [15] and Animasaun *et al.* [17], with few modifications in the time of fixation from 6 hr to 24 hr. The root tips were pretreated with Para-dichlorobenzene for four hours to arrest a large number of metaphase cells and to make the chromosomes more condensed for easy counting. The treated root tips were then extracted from the solution using a pair of forceps, rinsed twice with double-distilled deionised water, and subsequently transferred to a tube containing freshly prepared ethanol:glacial acetic acid (3:1, v/v) as a fixative for 24 h. The root tips were rinsed with ice-cold distilled water after being removed from the fixative. Following this, the tips were hydrolysed in a watch glass with 1 N HCl for 20 min at room temperature, after which the HCl was discarded. Next, the tips were transferred to clean microscopic slides, retaining 2-3 mm of the meristematic root tip, and the other root parts were cut off and discarded. The root meristem was stained with 1% (w/v) acetic carmine, left for 10 min. before being covered with a coverslip. The root tip was squashed in 45% acetic acid by pressing the slide between the thumb and index finger, producing a gentle tap until a turbid suspension was formed. After this, the slide was covered with a cover slip in preparation for microscopic observation using an Olympus microscope.

2.4 Scoring of Slides and Data Analyses

Slides were prepared to observe the effects of the *O. gratissimum* concentrations at different stages of mitosis. The slides were observed using a light microscope (Olympus), starting with the lowest objective lens ($\times 4$) and progressing steadily to the highest ($\times 40$). The mitotic index was calculated from the dividing and non-dividing cells observed under the microscope, captured digitally. Chromosome forms, aberrations or anomalies seen were captured at each stage of mitosis. Approximately 600 cells were observed for each treatment and scored for mitotic index (MI) and mitotic inhibition.

Cytotoxicity determination: a comparison was conducted to analyse the behaviour and morphology of chromosomes in cells exposed to *O. gratissimum* or without such exposure (control). For cells that display mitotic irregularities, decreased mitotic index, chromosome damage, or cell death, the *O. gratissimum* dosage was considered cytotoxic. Additionally, the presence of

micronuclei (MNs) was examined among the treatment groups. The mitotic index (MI) was determined according to Eq. (1) [18]. Chromosomal aberrations (CAs) refer to alterations in either the structure or total number of chromosomes (karyotypes) following exposure to a chemical agent. Mitotic inhibition was measured using the method of Fiskejo [19] Eq. (2). Representative micrographs documenting frequent abnormalities were produced.

$$\text{Mitotic Index} = \frac{\text{Dividing cells in all phases}}{\text{Total number of cells}} \quad (1)$$

Mitotic Inhibition (%) =

$$\frac{\text{Mitotic index of Control} - \text{Mitotic index of Test}}{\text{Mitotic index of Control}} \quad (2)$$

2.5 Statistical Analysis

Data obtained from the study were analysed using one-way analysis of variance (ANOVA), with Tukey's post-hoc test to determine significant means at $p < 0.05$. The analysis was conducted using SPSS version 22 (IBM, Armonk, USA), and the results are expressed as mean. The graphical representations for visualisation were achieved with Python 3.9.

3.0 Results

Root length decreased progressively with increasing concentrations of *O. gratissimum* extract. The highest inhibition of root growth was recorded at 100% concentration. These results show that all tested concentrations of *O. gratissimum* leaf extracts caused significant inhibition of root growth in comparison to the control (Figures 1 and 2). The inhibition of root number and root length was greater with increasing concentrations of *O. gratissimum* leaf extracts. The root morphology was nearly normal for the control treatment, but at 20% *O. gratissimum* leaf extract, the roots appeared slightly yellow. For the 40% and 60% *O. gratissimum* leaf extract concentrations, the roots are light brown. Meanwhile, 80% and 100% *O. gratissimum* leaf extract produced roots that were deep brown (Figure 3).

The mitotic index (MI) declined sharply across all extract concentrations (Table 1), with the control showing the highest MI (3.78%) and the 100% treatment the lowest (1.56%). This decline indicates a concentration-dependent mitotic suppression caused by the extract. Mitotic inhibition increased with extract concentration, signifying that even low levels of *O. gratissimum* can substantially suppress cell division.

Although normal cell division phases were observed in the treated roots (Figure 4), as

expected, cells at different stages of mitotic division were observed. Nevertheless, the cytological examination revealed dose-dependent chromosomal aberrations, including stickiness, bridges, laggards, vagrant chromosomes, and binucleated cells. The frequency of abnormalities increased with concentration, with the 80% and 100% treatments showing the highest incidences.

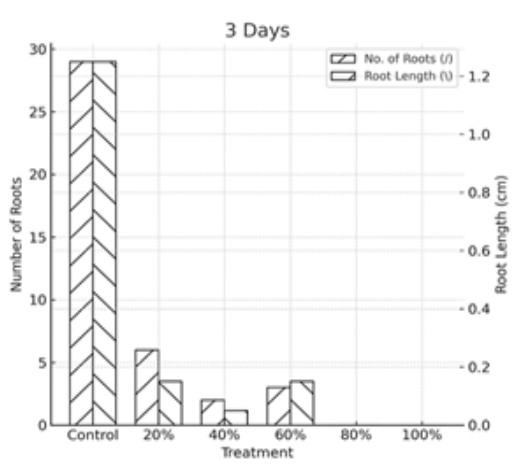


Figure 1: Variations in root numbers and root length (cm) of *Allium cepa* bulbs were observed under different concentrations of *O. gratissimum* extract on Day 3. The mean value of three replicates \pm SE is presented.

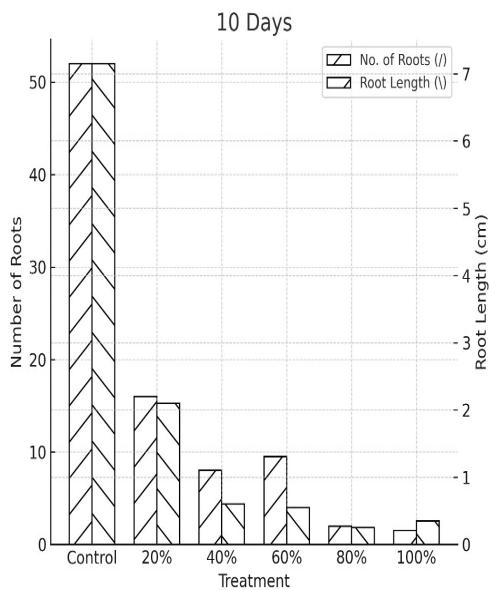


Figure 2: Variations in root numbers and root length (cm) of *Allium cepa* bulbs were observed under different concentrations of *O. gratissimum* extract at 10 days. The mean value of two replicates \pm SE is presented.



Figure 3: *Allium cepa* root formation in response to varying concentrations of *O. gratissimum* extract exposure at 10 days after treatment

4.0 Discussion

This study demonstrates that *O. gratissimum* aqueous extract exerts cytotoxic effects on *Allium cepa* root meristem cells in a dose-dependent manner. This is evident from the statistically significant reduction in root number and root length with increasing extract concentrations, along with observable chromosomal aberrations and mitotic suppression. The inhibition of root growth (both in terms of number and length) increased significantly with increasing extract concentration, with near-complete suppression observed at concentrations of 80% or higher. This aligns with findings of Fasola and Egunyomi [20], who reported that phytochemical-rich extracts can hinder mitotic progression and inhibit elongation due to impaired cell proliferation. Root tips exposed to higher concentrations exhibited discolouration, morphological abnormalities, and stunted growth, reflecting possible damage to cellular structures involved in elongation [21].

Interestingly, while root growth generally progressed over time in the control and lower-concentration groups, the extract-treated groups showed much slower or stagnant growth. This time-independent inhibition is consistent with the results of Animasaun *et al.* [6], suggesting that the toxicity of the extract overrides natural developmental progression. The mitotic index (MI) data provide strong evidence of antimitotic activity. The MI dropped from 3.78% in the control to 1.56% at 100% concentration of the extract; this is remarkable and sufficient to infer that the use of crude extract for herbal remedies may not be advisable. Thus, the results indicated that *O. gratissimum* extract contains bioactive compounds capable of arresting cell division, likely by interfering with spindle fibre formation or DNA synthesis [22, 23].

Table 1: Cytological effects of exposure to varying concentrations of *O. gratissimum* extract on *A. cepa* mitotic cells

Treatment (%)	Total Cells Counted	Number of Dividing Cells	Mitotic Index (%)	Mitotic Inhibition (%)
Control (0)	555.00	21.00	3.78	0.00
20	782.00	13.00	1.66	56.08
40	489.00	8.00	1.64	56.77
60	429.00	7.00	1.63	56.87
80	338.00	6.00	1.57	58.50
100	307.00	6.00	1.56	58.73

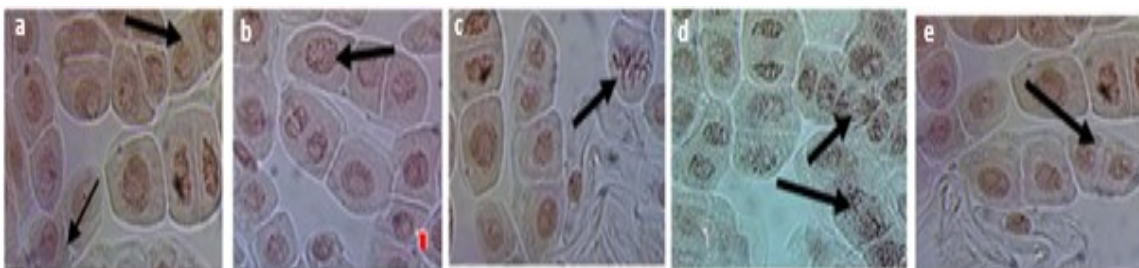


Figure 4: Photomicrographs of normal interphase and actively dividing cells in *Allium cepa* root tip meristematic region: a interphase; b prophase; c metaphase; d anaphase; and e telophase

Mitotic inhibition exceeding 50% in all treated groups further highlights the extract's potential to halt proliferation, possibly due to the presence of phytochemicals like eugenol, saponins, and flavonoids, which have been documented to exhibit mitostatic and genotoxic properties [24, 25].

Microscopic observations revealed a range of chromosomal abnormalities, including sticky chromosomes, anaphase bridges, chromosome fragmentation, lagging chromosomes, binucleated cells, and micronuclei. These anomalies increased in severity with concentration and are classic markers of genotoxic and clastogenic damage, signifying disruptions in chromatin structure and mitotic spindle function [26, 27]. Sticky chromosomes, observed in concentrations above 60%, often indicate DNA-protein cross-linking, a symptom of phytochemical-induced toxicity [24].

The presence of micronuclei at higher concentrations, particularly at 80-100%, suggests significant genomic instability and chromatin expulsion. This may be due to the presence of eugenol and other phytochemicals that impair spindle formation and DNA replication. The consistent cytotoxic pattern obtained from the mitotic data emphasizes the potential risks of unregulated exposure to *O. gratissimum* and supports its evaluation as a potent inhibitor of cell proliferation. Therefore, the cytological data demonstrated a clear mitotic suppression effect, characterised by a significant reduction in mitotic index and increasing mitotic inhibition with increasing extract concentrations. Thus, the effects

are indicators of genotoxins and have been validated using the *Allium cepa* assay [28, 29].

The *Allium cepa* test system remains a valuable tool for preliminary genotoxicity screening of plant-based substances due to its sensitivity, low cost, and correlation with mammalian systems [30, 31]. The findings here affirm its suitability in detecting chromosome aberrations and the consequential DNA-damaging potential of traditionally used medicinal plants. Therefore, folkloric and remedial uses of plant extracts, including *O. gratissimum*, should be carefully evaluated to avert possible associated risk of cytotoxicity.

5.0 Conclusion

This study demonstrated that aqueous leaf extract of *O. gratissimum* has significant cytotoxic effects on *Allium cepa* root meristem cells. Increasing extract concentrations resulted in fewer root numbers, reduced root length, suppressed mitotic index, and a broad spectrum of chromosomal and nuclear abnormalities. These effects suggest interference with the normal process of cell division, likely due to the presence of cytotoxic phytochemicals such as eugenol and alkaloids. Although *O. gratissimum* is widely used in traditional medicine, its unregulated use at high doses may pose cytotoxic and genotoxic risks, warranting careful consideration of dosage in both traditional and modern applications.

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Disclosure of conflict of interest

None

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None

Authors Contributions

DAA, and JEP: Conceptualisation and design of the research idea; SBA and JEP: Preparation and evaluation of the extracts; DAA, JEP, GOO, MDA, and YFA: Laboratory works, data accumulation and analysis, development of the manuscript draft; DAA, JEP, SBA, GOO, MDA, and YFA: Revision and development of the final manuscript.

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