EVALUATION OF GENOTOXIC AND CYTOTOXIC ACTIVITIES OF LEAF AND SEED EXTRACTS OF *Telfairia occidentalis*

Abstract

Telfairia occidentalis Hook (cucurbitaceae) leaves and seeds, which are used as vegetable in the preparation of soups and as medicine traditionally to treat various diseases by the Ibibios were investigated for genotoxic and cytotoxic effects using *Allium cepa* test. The effects of the leaves and seeds extracts on the root meristem cells of *Allium cepa* were investigated using onion bulbs exposed to 2.5 mg/mL, 5 mg/mL, and 10 mg/mL concentrations of the extracts 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 statistically significant ($p< 0.05$) inhibition of root growth depending on concentration by the extracts when compared with the negative control group. All the tested extracts were observed to have cytotoxic effects on cell division in *A. cepa.* The extracts-induced chromosomal aberrations and micronuclei (MNC) formations in *A. cepa* root tip cells were significant ($p<0.05$) when compared with control group. The extracts treatment further induced cell death, ghost cells, cells membrane damage, and binucleated cells. The seed extract was found to have a higher cytotoxic and genotoxic effects than the leaf extract. These results suggest that the leaf and seeds extract of *Telfairia occidentalis* possess cytotoxic and genotoxic effects on *A. cepa*.

Keywords: Genotoxic, Cytotoxic, Medicinal plants, Vegetables, *Telfairia occidentalis, Allium cepa.*

Introduction

Some phytochemical constituents of plants have the potentials to exert cytotoxic and genotoxic effects in biological systems. Studies have also demonstrated that a good number of plants used as food or medicine traditionally have the ability to cause mutagenic effect as well as cytotoxic and genotoxic effects *in vitro* and *in vivo* (Kassie *et al.*, 1996; As *et al.*, 2007; Johnny *et al* ., 2023; Okokon *et al.*, 2023a). These nutriceutic plants with potential mutagenic and/or carcinogenic constituents (Ames, 1986; Fernandes De S´a Ferreira and Ferr˜ao Vargas, 1999) have been linked with high rate of tumour formation in some human populations (Wynder *et al.*, 1983; Nagao *et al.*, 1986; Nguyen *et al.*, 1989; Brito *et al*., 1990). This information on the potential toxic hazards that may likely result from prolonged use of such plants especially these vegetables is very valuable. In Niger Delta region of Nigeria, the Ibibios use either domestic or wild vegetables in the preparation of soup. Examples of such vegetables are *Heinsia crinata* (atama), *Lasianthera africana* (editan), *Justicia insularis* (Memme) and *Telfairia occidentalis* (Ikong ubong). However, genotoxic and cytotoxic potentials of *H. crinata* , *L. africana* and *J. insularis* have recently been reported (Ikechukwu *et al*., 2024).

Telfairia occidentalis Hook is a fluted pumpkin of the *Cucurbitaceae* family widely consumed as food in Nigeria (Okokon *et al*., 2009). It is a popular vegetable all over Nigeria, especially in the Niger-Delta region and the Eastern part of the country; varieties of meals are prepared from the leaves, stem and seeds of the plant (Usunomena *et al.*, 2023). The seeds are very nutritious and are eaten roasted or boiled. The seed extract has been reported to exert antidiabetic (Eseyin *et al.*, 2007) , cellular antioxidant, immunodulatory, anticancer, antiinflammatory (Okokon *et al.*, 2012a), antiplasmodial (Okokon *et al.*, 2009), antioxidant (Osukoya *et al*, 2016),

analgesic (Okokon *et al.*, 2012b; Osukoya *et al*, 2016) and *in vivo* alpha amylase and alpha glucosidase inhibitory activities (Enin *et al.*, 2023). while the leaf extract possesses antioxidant, antibacterial (Oboh *et al.*, 2010), hepatoprotective (Nwanna and Oboh, 2007) antidiabetic (Nwozo *et al.*, 2004) and antiplasmodial (Okokon *et al.*, 2009) activities. Phytochemical studies of the extract have shown the presence of alkaloid, flavonoid, tannins, terpenes, saponin, and cardiac glycosides (Ebong *et al.*, 2020). Tannins, flavonoids, alkaloids, saponins, steroids, anthraquinones, and reducing sugars in the leaves have been reported. Long chain n-3-unsaturated fatty acid have been isolated from the leaf such as Palmitoleic acid and elaidic acid (Inuwa *et al.*, 2012). Okokon *et al*. (2012b) reported the presence of compounds such as pentadecanoic acid, hexadecanoic acid; 16-octadecenoic acid methyl ester; 9, 12-octadecadienoyl chloride (Z,Z); 9- Octadecadienoic acid (Z)-, 2, 3-dihydroxypropyl ester; Octadecanoic acid; hexadecanoic acid,2,3-is[(trimethylsilyl)oxy] propyl ester, 2,4-heptadien-6-ynal,(E,E); benzoic acid ; dodecanoic acid ; linoleic acid ethyl ester ; hexadecanoic acid, methyl ester ; α-phellandrene ; α-campholene aldehyde; terpinen-4-ol ; trans-β-ocimene ; borneol and stigmastan-3- ol, in the seed extract.

However, there is paucity of information on the genotoxic and cytotoxic effects of this vegetable. Therefore, this present study was designed to evaluate the leaves and seeds extracts of *T.occidentalis* for genotoxic and cytotoxic activities using *Allium cepa* test.

MATERIALS AND METHODS

Plant collection

Fresh leaves and seeds of *Telfairia occidentalis* were purchased from Itam market in Itu L. G. A, Akwa Ibom State, Nigeria, in June, 2023. The leaves and seeds were previously identified and authenticated by a taxonomist in the Department of

Botany, University of Uyo, Uyo, Nigeria. Herbarium specimens (UUPH 1(b)) were deposited at Department of Pharmacognosy and Natural Medicine Herbarium, University of Uyo.

The fresh leaves and seeds of the plant were dried on laboratory table for 2 weeks and reduced to powder. The leaves powder (1 kg) and seeds powder (1 kg) were separately macerated in 50% ethanol (5000 mL) for 72 hours. The liquid filtrates obtained were concentrated at 40˚C and all the ethanol was completely removed. The extracts were stored in a refrigerator at 4˚C until used for experiment reported in this study.

***Allium cepa* test.**

Small onions bulbs, *A. cepa*, were procured from Itam market, Itu, Akwa Ibom State, Nigeria. The bulbs were prepared for the experiment by removing the outer scales and the dry bottom plate without destroying the root primordia using a small sharp knife and collected in a water container. Distilled water (200 mL) was used to dissolve each of the extract (20 g) which were thereafter prepared and diluted to different concentrations of each extract 2.5 mg/mL, 5 mg/mL and 10 mg/mL respectively from the respective stock solutions. Test concentration of each plants' extract at 2.5 mg/mL, 5 mg/mL, and 10 mg/mL concentrations prepared in 15 mL beakers were arranged in a series of 5 per test concentration and filled up for each concentration. One *A. Cepa* bulb was placed on top of each beaker, with the root primordia downward toward the liquid. Tap water was used as negative control and Methotrexate (0.1 mg/mL) was used as positive control. After 24 hours, the test samples were changed in the controls and all test concentrations and photographs of the growing *A. cep*a roots were captured. This continued for 72 hours, after which the roots were counted per beaker in all the tested concentrations and mean root number

was calculated. Similarly, the roots' lengths were measured using a metre rule and the mean root length was calculated. These were also done for the control. Several root tips were cut at a length of 10 mm from the bulbs at 8:30 am, and respectively fixed in 3:1 (v/v) ethanol: glacial acetic acid and 1N HCL before putting them in sample bottles and storing in a refrigerator until use.

Microscopy

The root tips were each placed in a test tube with 1N HCL and heated at 50ºC for 6 minutes in order to fix and macerated them. Thereafter, the root tips were placed on microscopic slides on a blank background with forceps and were cut off at terminal tips. Two drops of 2% (w/v) orcein stain was added and mixed with the rootlets properly by knocking and stirring with a stirring spatula.

Then a cover slip was placed at 45º to avoid air bubbles. After that, the cells were squashed by placing a filter paper on the cover slip and pressed slight with a thumb. The cover slip was sealed with a clear finger nail polish and each slide was examined using a Light Microscope at a magnification of x40. Microphotographs were taken to show chromosomal aberrations. The mitotic index and frequency of chromosomal aberration were calculated based on the number of aberrant cells per total cells scored at each concentration of each sample (Bakare *et al.,* 2000). The mitotic inhibition was determined using the following formula:

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

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

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

The following parameters were used for determination 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 percentage and (i) chromatin aberrations (stickiness, bridges, breaks and polar deviation) were used as endpoints for determination of cytogenetic effects and micronuclei (MNC) were scored in interphase cells per 500 cells (Okokon *et al.*, 2023b).

Statistical Analysis.

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

RESULTS

Physicochemical Characterization.

The effects of leaf and seed extracts of *Telfairia occidentalis* on levels of the physicochemical parameters (root number and root length) are presented in Table 1. These results show that all tested concentrations of leaf and seed extracts of *Telfairia occidentalis* caused significant inhibition of roots growth in comparison to negative control and positive control. Increasing concentrations of the leaves and seeds extracts of *T. occidentalis* were found to cause corresponding inhibition of root number and root length. The average root length in negative and positive control (methotrexate) groups were 4.66±1.26 and 0.10±0.01 cm respectively. However, average root lengths in 10 mg/mL treatment groups of the two extracts were 0.52±0.22 and 0.38±0.09 cm respectively for leaves and seeds extracts of *Telfairia occidentalis*. These observed decreases in root lengths of the two extracts-treated

groups were significant ($p<0.05$) when compared to that of the negative control (Table 1). The average root lengths in treatment groups of the extracts decreased with increasing concentrations. The seeds extract was found to exhibit higher inhibitory potentials on the root growth than the leaf extract and these were significant ($p<0.05$) when compared to negative control. The root morphology of the negative control treatment group appeared to be normal, while 2.5 mg/mL treatment groups of the two extracts were slightly yellowish in appearance. The morphology of roots from 5 and 10 mg/mL treatment groups the two extracts appeared brownish (Table 1).

Cytogenetic Analysis.

Table 2 shows the effects of seed and leaf extracts of *T. occidentalis* on cytogenetic parameters of *Alium cepa* roots. Cytogenetic analysis performed showed that both extracts caused concentration-dependent and significant ($p<0.05$) decreases in the mitotic index when compared to that of negative control. Mitotic indices of 17.60±1.23 and 7.60±1.55 were respectively recorded for 10 mg/ml of seed and leaf extract of *T. occidentalis* as compared to 60.40±8.24 recorded in the negative control group (Table 2). This showed that the seed extract was more cytotoxic that the leaf extract of *T. occidentalis.*

Table 1: Cytotoxicity of *Telfairia occidentalis* leaf and seed extract on growing roots of Onion (*Allium cepa*)

Treatment group	Concentration of extract (mg/mL)	Average root Number ± S. D	Average root length (cm)± S.D
Negative control	Tap water	35.80±2.41	4.66±1.26
Methotrexate	0.1	2.10±0.02[a]	0.10±0.01[a]
Telfairia occidentalis Leaf extract	2.5	23.80±2.59[a]	0.80±0.08[a]
	5.0	15.80±0.58[a]	0.56±0.14[a]
	10.0	15.0±3.76[a]	0.52±0.22[a]
Telfairia	2.5	17.40±3.89[a]	0.54±0.10[a]

occidentalis seed extract	5.0	16.20±2.10[a]	0.42±0.05[a]
	10.0	10.80±1.24[a]	0.38±0.09[a]

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

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	302	60.4±8.24
Methotrexate	0.1	500	15	3.00±0.68[a]
Telfairia occidentalis Leaf extract	2.5	500	165	33.00±2.14[a]
	5.0	500	116	23.20±3.26[a]
	10.0	500	88	17.60±1.23[a]
Telfairia occidentalis Seed extract	2.5	500	86	17.20±1.15[a]
	5.0	500	80	16.00±1.42[a]
	10.0	500	38	7.60±1.55[a]

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

Cytogenetic alterations caused by the seed and leaf extracts of *Telfairia occidentalis* are shown in Table 3. Chromosome and cytological alterations were observed in negative control, methotrexate, seed and leaf of *T. occidentalis* extracts-treated groups as depicted in Table 3. Analysis of chromosome aberrations showed apoptotic bodies, membrane damaged cells and stickiness (Table 3; figures 1A). Binucleated cells were also seen (Figure 1B) as well as laggard and bridge chromosomes (Figures 1C, 1E and 1I). Polar deviations (wrong directions of chromosome movement) were observed only in the group treated with seed extract

(5.0 mg/mL) (Figure 1(D)). Fragments or clastogenic breaks of chromosomes were observed at higher concentrations of seed extract (5.0 and 10.0 mg/mL) (Table 3; Figure 1 (H)). This observation showed the clastogenic effect of the seed extract. This was significant ($p<0.05$) when compared to negative control group. Sticky metaphase and telophase were also observed in the seed and leaf extracts- treated groups (Figures 1(F and L)). These abnormalities were found to increased with increasing concentrations of the extracts generally especially in seed extract-treated groups. The number of aberrant cells (aberrant cells include chromosome breaks, stickiness and polar deviation) were found to be concentration-dependent and statistically significant ($p<0.05$) when compared to the negative control (Table 3; Figure 1). However, the highest value of aberrant cells was observed in methotrexate-treated group (positive control) (Table 3). Genotoxic potentials of the extracts were further demonstrated by the induction of micronuclei in the root tip meristem cells of *A. cepa*. Micronucleus formation in 500 cells per slide (‰MNC value) was not concentration-dependent but the frequency was higher in leaf extract-treated groups than the groups treated with the seed extract. The leaf extract-treated groups had high numbers of cells with micronuclei in the test compared to negative control and these were statistically significant ($p< .05$). Also, cells with nuclear and membrane damage (Figures 1(H, J and L)), binucleated cells (Figure 1(B)), ghost cells 1(G)) and apoptotic cells (Figure 1(A)) were found in various frequencies in the groups treated with the two leaf extracts.

Table-3: Chromosomal and mitotic aberrations in the root meristematic cells of *Allium cepa* after treatment with *Telfairia occidentalis* leaf and seed extracts

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	-	0.04±0.02	-	4.00±0.34	-
Methotrexate	0.10	2.34±1.23 [a]	21.34±5.38 [a]	10.55±2.28 [a]	45.13±4.22 [a]	2.28±0.86 [a]
Telfairia occidentalis Leaf extract	2.5	-	1.13±0.49[a]	-	21.34±1.24[a]	2.25±0.22[a]
	5.0		2.16±10.68[a]	-	30.19±5.29[a]	2.15±0.14[a]
	10.0	-	18.24±3.26[a]	-	44.12±3.84[a]	2.14±0.34[a]
Telfairia occidentalis seed extract	2.5	-	2.06±0.23 [a]	-	31.25±2.48[a]	0.16±0.01[a]
	5.0	2.25±0.18[a]	5.32±1.50[a]	0.29±0.01[a]	38.02±3.36[a]	0.05±0.01[a]
	10.0	4.20±0.24[a]	12.34±3.26[a]	-	40.88±4.18[a]	0.13±0.0.2[a]

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

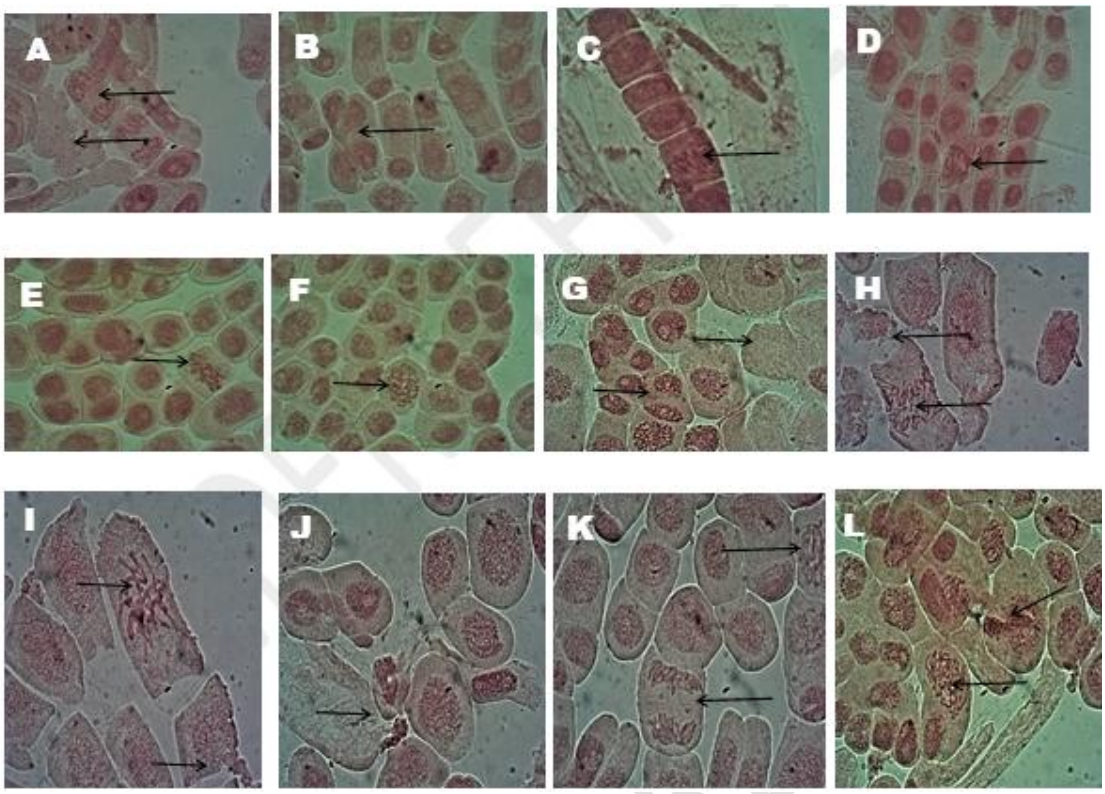

Figure 1: Photomicrograph showing the mitotic and chromosomal aberrations of *Allium cepa* root meristem cells after leaf and seed extracts of *Telfairia occidentalis* treatment under light microscope X40 magnification. (A) arrows indicate Stickiness and apoptotic bodies (B) binucleated cells (C) Bridge and laggard chromosomes (D) Polar deviation (E)Laggard chromosomes (F) Sticky chromosomes (G) Ghost cells and binucleated cells (H) nuclear damage and fragmentation (I) Bridge (J) Membrane and nuclear damage (K) Sticky Telophase (L) Sticky chromosomes and nuclear damage

Discussion

In this study, toxic effects of seed and leaf extracts of *Telfairia occidentalis* were evaluated on root growth and root morphology of *Allium cepa*. Different concentrations of the extracts (2.5 -10.0 mg/mL) employed were observed to cause inhibition of root growth and these were statistically significant when compared to control group. Moreover, the seed and leaf extracts caused colouration of the root tips of *Allium cepa* which ranged from yellow to dark brown coloration of the roots, depending on the concentration. The cyto- and genotoxicity effects were investigated by assessing cytological parameters such as the mitotic index and number of chromosome abnormalities, including chromosome breaks, stickiness, and polar deviations. The mitotic index (MI) of *A. cepa* meristematic cells

treated with methotrexate (0.1 mg/mL) was significantly decreased when compared to control. Also, significant inhibition in the onion roots treated with the *Telfairia occidentalis* seed extract had mitotic indices of 33.00%, 23.20% and 17.20% for 2.5, 5.0 and 10.0 mg/mL respectively, while the leaf extract had mitotic indices of 17.20%, 16.00% and 7.60% respectively for 2.5, 5.0 and 10.0 mg/mL (Table 2). These were significant when compared to control. The root growth inhibition correlated with decreased of mitotic Index. Serious toxic effect on the organism is indicated when the mitotic index value falls below 22% when compared to negative control (Antonsie-Wiez, 1990), while a value below 50% usually indicates sublethal effects (Panda and Sahu, 1985) and is called cytotoxic limit value (Sharma, 1983). Mitotic index measures the proportion of cells in the M-phase of the cell cycle and its inhibition could be interpreted as cellular death or a delay in the cell proliferation kinetics (Rojas *et al.*, 1993). Reduction in the mitotic activity could be attributed to inhibition of DNA synthesis or a blocking in the G2 phase of the cell cycle, preventing the cell from entering mitosis (Sudhakar *et al.*, 2001). Mitotic depressive effects of some herbal extracts, which could be due to blockage of DNA synthesis and nucleus proteins, have been reported previously (Mercykutty and Stephen, 1980; Schulze and Kirschner, 1986; `As *et al.*, 2007; As *et al*., 2006; Akinboro and Bakare, 2007). The decreased mitotic indices in *A. cepa* roots treated with the seed and leaf extracts could have resulted from their potentials to cause disturbances in the cell cycle or chromatin dysfunction due to extracts-DNA interactions. The results of this study suggest that the tested extracts concentrations have inhibitory, mito-depressive effects on root growth and cell division of *A. cepa.* Thus, it can prevent DNA synthesis and cause reduction in the number of dividing cells in the roots, due to the cytotoxic effects of compounds found in the extracts. The observation of sticky metaphase demonstrated the toxic effect of the extracts and this was common with all groups treated with the two

extracts. Metaphases with sticky chromosome, loses their normal appearance, and present with a sticky "surface," causing chromosome agglomeration (Babich *et al*., 1997). Stickiness has been attributed to the effect of pollutants and chemical compounds on the physical-chemical properties of DNA, protein or both, on the formation of complexes with phosphate groups in DNA, on DNA condensation or on formation of inter- and intra-chromatid cross links (Gomurgen, 2005). 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 (Swierenga *et al*., 1991). The presence of chromosome fragments is an indication of chromosome breaks, and can be a consequence of anaphase/telophase bridges (Sharma and Sen, 2002). Fragments were observed in this study especially in the seed extract treated groups. The seed extract was found to not only interfere with the cell cycle, but also affect chromatin organization or DNA replication, causing chromosome breaks. Polar deviation was observed in seed extract (5.0 mg/mL) treated groups. Frequencies of total chromosome aberrations increased significantly following exposure to the extracts which indicate clastogenic activity (Table 3). The extracts significantly induced the formation of MNC in *A. cepa* root cells at 2.5–10 mg/mL concentrations. Frequencies of MNC were found to be higher in the groups treated with the leaf extract. However, MNC frequency decreased in *A. cepa* roots treated at the highest concentration of the extracts (10 mg/mL), due to high cytotoxicity. The micronuclei frequency of cells is a good indicator of the cytogenetic effects of tested chemicals. Micronuclei (MN) often results from the acentric 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 (Albertini *et al.*, 2000; Krishna and Hayashi, 2000). Previous studies have suggested

MNC-induced effect of various plant extracts such as *Lavandula stoechas* and *Ecballium elaterium* (As *et al.,* 2007; As *et al.,* 2009), *Azadirachta indica* (Soliman, 2001), *Psychotria species* (Akinboro and Bakare, 2007).

In this study, membrane damage cells were observed in groups treated with various concentrations of the seed and leaf extracts 2.5, 5.0 and 10.0 mg/mL but mostly in the seed extract -treated groups. These results show that the extracts over certain concentrations may cause cytotoxicity as they cause membrane damage. These results suggest the cytotoxic potentials of the extracts. Binucleated cells were observed in extracts-treated groups. This is due to the prevention of cytokinesis or cell plate formation. Microtubules are involved in cell plate formation and the extracts inhibit the process, resulting in inhibition of cytokinesis. Ghost cell is a dead cell in which the outline remains visible, but whose nucleus and cytoplasmic structures are not stainable (As *et al.,* 2009). Some ghost cells were observed in various frequencies in this study in both extracts but mostly in the seed extract (10 mg/mL) treated groups (Figure 1(G). This could have resulted from the activities of the phytochemical constituents of the extracts leading to nucleus damage and prevention of cytoplasmic structures, thus resulting in ghost cells. In addition, the extracts also induced DNA damage and cell death and/or apoptosis in various frequencies in this study. In this study, high concentrations of the extracts were observed to cause the induction of cell death and/or apoptosis. Cell death is a basic biological process of living organism. The cell death is induced by high concentrations of such as toxin, stress, heavy metals, chemicals and others.

The results of this study show that the seed and leaf extracts of *T, occidentalis* can induced cytogenetic alterations (cytoplasmic shrinkage, nuclear condensation, DNA fragmentation, membrane blebbing, cytoskeleton alterations and appearance of apoptotic

bodies) and cell death in root tips of *A. cepa* (Figures 1(A -P)) suggesting cytotoxic and genotoxic activities of the extracts.

Researches have revealed the phytochemical constituents of both extracts. Alkaloid, flavonoid, tannins, terpenes, saponin, and cardiac glycosides (Ebong *et al.*, 2020) as well as long chain n-3-unsaturated fatty acid such as palmitoleic acid and elaidic acid have been isolated from the leaves (Inuwa *et al.,* 2012). Okokon *et al*. (2012b) reported the presence of compounds such as pentadecanoic acid, hexadecanoic acid; 16-octadecenoic acid methyl ester; 9, 12-octadecadienoyl chloride (Z,Z); 9- Octadecadienoic acid (Z)-, 2, 3-dihydroxypropyl ester; Octadecanoic acid; hexadecanoic acid,2,3-is[(trimethylsilyl)oxy] propyl ester, 2,4-heptadien-6-ynal,(E,E); benzoic acid; dodecanoic acid; linoleic acid ethyl ester; hexadecanoic acid, methyl ester ; α-phellandrene ; α-campholene aldehyde; terpinen-4-ol; trans-β-ocimene; borneol and stigmastan-3- ol, in the seed extract. Polyunsaturated fatty acids have been reported in several studies to exert genotoxic and cytotoxic effects (Xu and Qian, 2014; Villa-Vasquez *et al.,* 2018; Giordano *et al.,* 2020; Kizilsahin *et al.,* 2022). their presence in the leaf and seed extracts among other constituents may have been responsible for the observed effects in this study.

In addition,the phenolic and flavonoid contents as well as terpenoids compounds in the seed and leaf extracts may have been responsible for the observed effects in this study. Quercetin, a flavonoid and others flavonoids have been reported to demonstrate mutagenic and genotoxic potentials in various studies (Ping *et al*., 2017) and these compounds are present in the extracts of this vegetable thereby accounting for the observed activities. These findings explain the mechanism of action and corroborate earlier report of Okokon *et al.,* (2012a) on the anticancer activities of the seed extract and fractions. Thereby, laying credence to the use of the seed of *T. occidentalis* in traditional medicine for the treatment of prostate cancer. The high phenols and flavonoids contents in

the leaf extract must have contributed to the observed cytotoxic and genotoxic activities in this study.

Conclusion

This study revealed that the seed and leaf extract of *Telfairia occidentalis* possess cytotoxic and genotoxic effects with the seed extract having a higher potency as seen in the effects elicited by all test concentrations of the two extracts on the root number, root length and root morphology of the *Allium cepa* meristems after exposure. The degree of chromosomal aberrations (based on increasing extract concentration), the inhibition of cellular mitotic processes, and the general abnormalities observed in all root bulbs treated with test samples further indicate cytotoxic potentials of the plant.

References

Akinboro A. and Bakare, A. A. (2007). Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants," *Journal of Ethnopharmacology*, 112(3):470–475.

Albertini, R. J., Anderson, D., Douglas, G. R. (2000). IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans," *Mutation Research*, 463(2):111–172.

Ames, B.N (1986). "Food constituents as a source of mutagens, carcinogens and anticarcinogens," in *Genetic Toxicology of the Diet*, I. Knudsen, Ed., Alan R. Liss, New York, NY, USA, pp. 55–62.

Antonsie-wiez, D. (1990). Analysis of the cell cycle in the root of *Allium cepa* under the influence of Leda krin," *Folia Histochemica et Cytobiologica*, 26: 79–96.

As T., kınelik C¸ and Aslanturk, O. S. (2007). "Cytotoxic and genotoxic effects of *Lavandula stoechas* aqueous extracts," *Biologia*, 62(3): 292–296.

As¸ T. A., kin C¸ elik and Aslantu¨rk, O. S. (2009). Investigation of cytotoxic and genotoxic effects of *Ecballium elaterium* juice based on *Allium* test," *Methods and Findings in Experimental and Clinical Pharmacology*, 31(9):591–596.

As¸ T., kin C¸ elik and Aslantu¨rk, O¨. S. (2006). Anti-mitotic and antigenotoxic effects of *Plantago lanceolata* aqueous extract on *Allium cepa* root tip meristem cells," *Biologia*, 61(6): 693–697.

Babich, H., Segall, M. A. and Fox, K. D. (1997). "The Allium test— a simple, eukaryote genotoxicity assay," *American Biology Teacher*, 59(9): 580–583.

Bakare, A.A., Mosuro, A.A., Osibanjo, O., (2000). Effect of simulated leachate on chromosomes and mitosis in roots of *Allium cepa* L. *J. Environ. Biol.* 21, 263–271.

Brito,M. T., Martinez, A. and Cadavid,N. F. C.(1990). "Mutagenic activity in regional foods and beverages from the Venezuelan Andean region," *Mutation Research*, 243(2):115– 120.

Ebong AS, Eseyin OA, Etim EI, Okokon JE, (2020). *Telfairia occidentalis* potentiates antiplasmodial activity of artemisinin and amodiaquine combination therapy. *Anti-Infective Agents*. 18(2): 152-159.

Enin GN, Okokon JE, Odokwo BO, Antia BS. (2023).Preliminary phytochemical screening and i*n vivo* Inhibitory study of *Telfaira occidentalis* Hook f. seeds extract on alpha amylase and alpha glucosidase of rats. *Journal of Science and Technology Research* 5(4) : 26-35.

Eseyin OA, Ebong P, Ekpo A, Igboasoiyi A, Oforah E. (2007). Hypoglycemic effect of the seed extract of *Telfairia occidentalis* in rat. *Pak J Biol Sci* 10(3): 498- 501.

Fernandes De S´a Ferreira, I. C and Ferr˜ao Vargas, M. (1999). Mutagenicity of medicinal plant extracts in *Salmonella*/microsome assay," *Phytotherapy Research*, 13(5): 397–400.

G¨om¨urgen, A. N. (2005). Cytological effect of the potassium metabisulphite and potassium nitrate food preservative on root tips of *Allium cepa* L. *Cytologia*, 70(2):119– 128.

Giordano C, Plastina P, Barone I, Catalano S, Bonofiglio D. (2020). *n*–3 Polyunsaturated fatty acid amides: New avenues in the prevention and treatment of breast cancer. *International Journal of Molecular Sciences*. 21(7):2279.

Ikechukwu E, Agu P, Olumuji H, Anaboso MO, Ebong NO, Okokon JE. (2024).Evaluation of genotoxic and cytotoxic activities of three vegetables (*Heinsia crinata, Justicia insularis a*nd *Lasianthera africana*) Using *Allium cepa* Test. *Asian Journal of Biochemistry, Genetics and Molecular Biology* 16(4): (In Press)

Inuwa HM, Aimola IA, Muhammad A, Habila N, Okibe P, Latayo M, Ahmed Z. (2012). Isolation and determination of omega-9 fatty acids from *Telfairia occidentalis. Intl J Food Nutr Safety* 1(1): 9-14.

Johnny II, Okokon JE, Ochigbo EB, Udo IJ, Adefabi AM. (2023). Genotoxic and cytotoxicity potentials of *Hippocratea africana. Asian Journal of Biochemistry, Genetics and Molecular Biology*. 15(2): 38-45.

Kassie, F., Parzefall, W., Musk, S. (1996). Genotoxic effects of crude juices from *Brassica* vegetables and juices and extracts from phytopharmaceutical preparations and spices of cruciferous plants origin in bacterial and mammalian cells," *Chemico-Biological Interactions*, 102(1): 1–16.

Kizilsahin, S., Koksal Karayildirim, C., Bakan, B., Nalbantsoy, A., Karabay Yavasoglu N. U. (2022). Cytotoxicity and genotoxicity evaluations of oleic acid and conjugated linoleic acid. *İstanbul Journal of Pharmacy,* 52(1), 14-19.

Krishna, G. and Hayashi, M. (2000). *In vivo* rodent micronucleus assay: protocol, conduct and data interpretation," *Mutation Research*, 455(1-2): 155–166.

Mercykutty V. C. and Stephen, J. (1980). Adriamycin induced genetic toxicity as demonstrated by *Allium cepa* test," *Cytologia*, 45(4): 769–777.

Nagao, M., Wakabayashi, K., Fujita, Y., Tahira, T., Ochiaia, T. and Sugimura, T. (1986). Mutagenic compounds in soy sauce, Chinese cabbage, coffee, and herbal teas," in *Genetic Toxicology of the Diet*, I. Knudsen, Ed., Alan R. Liss, New York, NY, USA, pp. 55–62.

Nguyen, T., Fluss, L., Hodej, R., Ginther, G., and Leighton, T. (1989). "The distribution of mutagenic activity in red rose and white wines," *Mutation Research*, 223:205–212.

Nwanna E, Oboh G. (2007). Antioxidant and hepatoprotective properties of polyphenol extracts from *Telfairia occidentali*s (Fluted Pumpkin) leaves on acetaminophen induced liver damage. *Pak J Biol Sci* 10(16): 2682-2687.

Nwozo SO, Adaramoye OA, Ajaiyeoba EO. (2004). Anti-diabetic and hypolipidemic studies of *Telfairia occidentalis* on alloxan induced diabetic rabbits. *Nig J Nat Prod Med* 8: 45-47

Oboh G, Nwanna E, Elusiyan C. (2010). Antioxidant and antimicrobial properties of *Telfairia occidentalis* (Fluted pumpkin) leaf extracts. *J Pharmacol Toxicol* 5(8): 539-547.

Okokon JE, Ekpo AJ , Eseyin OA (2009)**.** Evaluation of in vivo antimalarial activities of ethanolic leaf and seed extracts of *Telfairia occidentalis.Journal of Medicinal Food,* 12(3):649-653.

Okokon JE, Antia BS, Dar A., Choudhary MI. (2012a). Immunomodulatory, anticancer and antiinflammatory activities of *Telfairia occidentalis* seed extract and fractions. *International Journal of Food Nutrition and Safety* 2(2): 72 - 85.

Okokon JE, Dar A., Choudhary MI. (2012b). Chemical constituents and analgesic activity of *Telfairia occidentalis*. Phytopharmacology 3(2): 359 – 366.

Okokon JE, Ochigbo EB, Johnny I, Anagboso MO, Ebong NO. (2023a). Genotoxic and cytotoxicity activities of *Setaria megaphylla. Asian Journal of Biochemistry, Genetics and Molecular Biology.* 15(3):56-64.

Okokon JE, Edet E, Etuk IC, Johnny II, Udo IJ. (2023b). Genotoxic potentials, total flavonoid and total phenolic contents of S*olanum anomalum*. *Asian Journal of Biochemistry, Genetics and Molecular Biology*.15(3): 104 -113.

Osukoya OA, Adegbenro D, Onikanni SA, Ojo OA, Onasanya A. (2016). Antinociceptive and antioxidant activities of the methanolic extract of *Telfairia occidentalis* Seeds. *Anc Sci Life.* 36(2):98-103.

Panda B. B. and Sahu, U. K. (1985). Induction of abnormal spindle function and cytokinesis inhibition in mitotic cells of *Allium cepa* by the organophosphorus insecticide fensulfothion," *Cytobios*, 42(167-168): 147–155.

Ping KY, Shohaimi S, Sasidharan S, Yusuf UK. (2017). Genotoxicity of selected chinese medicinal plants, *Elephantopus scaber*, *Glycyrrhiza uralensis and Salvia miltiorrhiza* on *Allium cepa* Assay. *Ann Pharmacol Pharm.* 2(13): 1070.

Rojas, E., Herrera, L. A., Sordo, M. (1993). Mitotic index and cell proliferation kinetics for identification of antineoplastic activity," *Anti-Cancer Drugs*, 4(6): 637–640.

Schulze E, Kirschner, M. (1986). "Microtubule dynamics in interphase cells," *Journal of Cell Biology*, 102(3):1020–1031

Sharma A. and Sen, S. (2002). *Chromosome Botany*, Science, Enfield, NH, USA.

Soliman, M. I. (2001). Genotoxicity testing of neem plant (*Azadirachta indica* A. Juss.) using the *Allium cepa* chromosome aberration assay," *Journal of Biological Sciences*, 1(11):1021–1027.

Sudhakar, R., Ninge Gowda, K. N. and Venu, G. (2001). Mitotic abnormalities induced by silk dyeing industry effluents in the cells of *Allium cepa*," *Cytologia*, 66(3):235–239.

Swierenga, S. H. H., Heddle, J. A., Sigal, E. A. (1991). 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," *Mutation Research*, 246(2):301–322.

Villa-Vasquez, S. S., Marquez-Fernandez, M. E., Camargo-Guerrero, M. (2018). Cytotoxic and genotoxic activity of omega fatty acids in prostate cancer cells PC-3. *Iatreia* 31(4):351-361.

Wynder,E. L., Hall, N. E. L. and Polansky, M.(1983). Epidemiology of coffee and pancreatic cancer," *Cancer Research*, 43(8): 3900–3906.

Xu Y, Qian SY. (2014). Anti-cancer activities of ω-6 polyunsaturated fatty acids. *Biomed J.* 37(3):112-9.