

EVALUATION OF GENOTOXIC AND CYTOTOXIC ACTIVITIES OF THREE VEGETABLES (*Heinsia crinata*, *Justicia insularis* and *Lasianthera africana*) USING *Allium cepa* TEST

ABSTRACT

Heinsia crinata (Afzel.) G. Taylor (Rubiaceae), *Justicia insularis* T. Anderson (Acanthaceae family) and *Lasianthera africana*. P.Beav (Stemonuraceae), which are used as vegetables 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 leaf extracts on the root meristem cells of *Allium cepa* were investigated using onion bulbs exposed to 2.5 mg/mL, 5mg/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. These results suggest that the leaf extracts of *Lasianthera africana*, *Heinsia crinata* and *Justicia insularis* possess cytotoxic and genotoxic effects on *A. cepa*.

Keywords: *Allium cepa*; Cytotoxic; Genotoxic; Medicinal plants; Vegetables

1. INTRODUCTION

"A number of green plants contain phytoconstituents which may exert cytotoxic and genotoxic activities due to their effects within a biological system. Researches have also shown that a number of plants which are utilised as food or medicine traditionally have mutagenic effects as well as cytotoxic and genotoxic effects in vitro and in vivo" [1, 2, 3, 4]. "Plants with potential mutagenic and/or carcinogenic substances which are used as food or medicine have been correlated with high rate of tumour formation in some human populations"[5, 6, 7, 8, 9, 10]. This reveals potential toxic hazards that may likely result from prolonged use of such plants especially these vegetables. 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) and *Justicia insularis* (Memme).

Heinsia crinata (Afzel.) G. Taylor (Rubiaceae), *Lasianthera africana*. P.Beav (Stemonuraceae) and *Justicia insularis* T. Anderson (Acanthaceae family) are also utilized in Ibibio traditional medicine for the treatment of a number of diseases such as malaria, diabetes, inflammation, pains, ulcer [11, 12], digestive, weaning agent, laxative [13, 14, 15, 16] among others. Reports of their biological activities as antimalarial [17, 12] antimicrobial [18, 19], antiulcer [11, 20], anticonvulsant [21], antidiabetic and hypoglycaemic [12, 22, 23, 24], analgesic and antipyretic [25, 26], immunomodulatory [27], anti-oxidant activity [16], and antianaemic [28] are published in literatures. Two triterpenoid saponins have been isolated from the leaves of *H. crinata* [29] and two iridoids; lamalbid 6, 7, 8- triacetate and aglycone lamiridosin 6, 7, 8-triacetate have also been isolated from the stem bark [30]. "Quercetin, quercetin 3-methyl ether, luteolin, rutin, quercetin-3-O-rutinoside (rutin), α -L-rhamnopyranosyl-(1-6)- β -D-glucopyranose) have been isolated and identified from the leaves of *L. Africana*" [31]. "Arylnaphthalide lignans and triterpenoid glycosides are indicated as the major types of chemical constituents of *J. insularis*"[32].

However, there is paucity of information on the genotoxic and cytotoxic effects of these vegetables. Therefore, this present study was designed to evaluate the leaves extracts of *H. crinata*, *L. africana* and *J.insularis* for genotoxic and cytotoxic activities using *Allium cepa* test.

2. MATERIAL AND METHODS

2.1 Plant materials

Fresh leaves of *Justicia insularis*, *H. crinata* and *L. africana* were procured from Itam market in Uyo, Akwa Ibom State, Nigeria, in May, 2023. The plants were previously identified and authenticated by a taxonomist in the Department of Botany, University of Uyo, Uyo, Nigeria. Herbarium specimens were deposited at Department of Pharmacognosy and Natural Medicine Herbarium. The fresh leaves (2 kg) of each plant were dried on laboratory table for 2 weeks and reduced to powder. The leaves powder (500 g) from each plant was macerated in 50% ethanol (5000 mL) for 72 hours. The liquid filtrate obtained was concentrated in vacuo 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.

2.2 *Allium cepa* test.

Small bulbs of the common onion, *A. cepa*, were procured from Elele market, Elele, Rivers State, Nigeria. Prior to initiating the test, the outer scales of the bulbs and the dry bottom plate were removed without destroying the root primordia using a small sharp knife and collected in a jar of water. Each of the plant extract (20 g) was dissolved in 200 mL of distilled water. Different concentrations of each extract 2.5 mg/mL, 5 mg/mL and 10 mg/mL respectively were prepared 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. cepa* 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[33].

2.3 Microscopy

The root tips were each placed in a test tube with 1N HCL and heated at 50°C for 6 minutes in order to fix and macerated them. Thereafter, the root tips were placed on microscopic slides on a blank background with a forcep 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 45° 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"[34]. 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 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 (ii) 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.

2.4 Statistical Analysis

Data obtained from this work were analyzed 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 i.e. $P \leq .05$.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Analysis

The effects of leaf extracts of *J. insularis*, *H. crinata* and *L. africana* on levels of the physicochemical parameters (root number and root length) are presented in Table 1. These results show that all tested concentrations of *J. insularis*, *H. crinata* and *L. africana* leaf extracts caused significant inhibition of roots growth in comparison to negative control and positive control. The inhibition of root number and root length increased with increasing concentrations of the leaf extracts. 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 three extracts were 0.30 ± 0.05 , 0.30 ± 0.03 and 0.40 ± 0.07 cm respectively for *J. insularis*, *H. crinata* and *L. africana*. These demonstrated decreases 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. *J. insularis* and *H. crinata* leaf extracts with comparable inhibitory potentials exhibited higher inhibition of the root growth than *L. africana* and these was 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 three extracts appeared slightly yellow. The morphology of roots from 5 and 10 mg/mL treatment groups the three leaf extracts appeared brownish (Table 1).

3.2 Cytogenetic Analysis.

Table 2 shows the effects of *J. insularis*, *H. crinata* and *L. africana* leaf extracts on cytogenetic parameters of *Allium cepa* roots. Cytogenetic analysis performed showed that the three leaf extracts caused concentration-dependent and significant ($p < 0.05$) decreases in the mitotic index when compared to that of negative control. The leaf extracts of *J. insularis*, *H. crinata* and *L. africana* at 10 mg/mL had mitotic indices of 10.48 ± 2.35 , 14.80 ± 2.34 and 11.0 ± 2.01 respectively as compared to 60.40 ± 8.24 recorded in the negative control group (Table 2). This showed that *J. insularis* was more cytotoxic followed by *L. africana* and *H. crinata*.

Table 1. Cytotoxicity of *Heinsia crinata*, *Justicia insularis* and *Lasianthera africana* leaf extract on growing roots of Onion (*Allium cepa*)

Treatment group	Concentration of extract (mg/mL)	Average root Number \pm S.D	Average root length (cm) \pm S.D
Negative control	Tap water	35.80 \pm 2.41	4.66 \pm 1.26
Methotrexate	0.1	2.10 \pm 0.02 ^a	0.10 \pm 0.01 ^a
<i>Justicia insularis</i>	2.5	16.60 \pm 2.42 ^a	0.84 \pm 0.21 ^a
	5.0	16.60 \pm 4.47 ^a	0.44 \pm 0.05 ^a
	10.0	8.00 \pm 1.80 ^a	0.30 \pm 0.05 ^a
<i>Heinsia crinata</i>	2.5	8.60 \pm 2.42 ^a	0.56 \pm 0.12 ^a
	5.0	14.20 \pm 4.25 ^a	0.44 \pm 0.04 ^a
	10.0	15.0 \pm 4.02 ^a	0.30 \pm 0.03 ^a
<i>Lasianthera africana</i>	2.5	4.40 \pm 0.97 ^a	0.60 \pm 0.04 ^a
	5.0	14.20 \pm 2.20 ^a	0.52 \pm 0.03 ^a
	10.0	25.0 \pm 5.25 ^a	0.40 \pm 0.07 ^a

Values are expressed as mean \pm SEM (n=5). Significant at $P < .05$ 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 (%) \pm S.E
Negative control	Tap water	500	302	60.4 \pm 8.24
Methotrexate	0.1	500	15	3.00 \pm 0.68 ^a
<i>Justicia insularis</i>	2.5	500	146	30.62 \pm 6.34 ^a
	5.0	500	86	15.40 \pm 4.14 ^a
	10.0	500	28	10.48 \pm 2.35 ^a
<i>Heinsia crinata</i>	2.5	500	280	32.60 \pm 5.25 ^a
	5.0	500	86	19.0 \pm 6.64 ^a
	10.0	500	28	14.80 \pm 2.34 ^a
<i>Lasianthera africana</i>	2.5	500	141	28.20 \pm 6.54 ^a
	5.0	500	64	12.8 \pm 1.29 ^a
	10.0	500	55	11.0 \pm 2.01 ^a

Values are expressed as mean±SEM (n=5). Significant at $P < .05$ when compared to negative control

Cytogenetic alterations caused by the leaf extracts are shown in Table 3. Chromosome and cytological alterations were observed in negative control, methotrexate, *J. insularis*, *H. crinata* and *L. africana* leaf extracts-treated groups as depicted in Table 3. Chromosome aberrations analysis showed that the fragments or clastogenic breaks observed in this study were at lower concentrations of *Heinsia crinata* (2.5 and 5.0 mg/mL) and were of chromosome type (Table 3; Figure 1 (H)). The observation showed the clastogenic effect of *H. crinata* leaf extract. This was significant ($p < 0.05$) when compared to negative control group. Polar deviations (wrong directions of chromosome movement) were observed only in *L. africana* leaf extract-treated groups especially in the group treated with 5.0 mg/mL concentration of *L. africana* (Figure 1(F)). Sticky metaphase and telophase were also observed in the leaf extracts- treated groups (Figure 1(E and K)). These abnormalities were found to increased with increasing concentrations of the extracts generally especially in *J. insularis*-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 as the groups treated with methotrexate and *Justicia insularis* leaf extract (2.5 and 10.0 mg/mL) followed by *L. africana* (5.0 mg/mL) had high numbers of cells with micronuclei in the test compared to negative control, which were statistically significant ($p < .05$) (Figure 1 (O)). Also, cells with membrane damage (Figure 1(D and L)) and nucleus damage (Figures 1(G and I)), binucleated cells (Figure 1(P)), ghost cells 1(C)) and apoptotic cells (Figure 1(J)) were found in various frequencies in the groups treated with the three leaf extracts

Table 3. Chromosomal and mitotic aberrations in the root meristematic cells of *Allium cepa* after treatment of extract of *Heinsia crinata*, *Justicia insularis* and *Lasianthera africana*

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
<i>Justicia insularis</i>	2.5	-	4.82±1.34 ^a	-	25.66±3.29 ^a	5.22±0.56 ^a
	5.0	-	4.24±2.04 ^a	-	33.48±4.36 ^a	1.64±0.02 ^a
	10.0	-	20.19±2.58 ^a	-	41.24±2.35 ^a	4.20±0.38 ^a
<i>Heinsia crinata</i>	2.5	1.20±0.16 ^a	6.02±1.15 ^a	-	25.12±3.28 ^a	1.53±0.03 ^a
	5.0	1.04±0.20 ^a	7.20±1.78 ^a	-	36.80±3.22 ^a	1.27±0.16 ^a
	10.0	-	16.40±4.52 ^a	-	44.02±5.81 ^a	2.54±0.44 ^a
<i>Lasianthera africana</i>	2.5	-	6.40±0.51 ^a	-	38.40±2.48 ^a	1.24±0.15 ^a
	5.0	0.14±0.02 ^a	12.83±2.58 ^a	5.33±1.32 ^a	48.03±6.39 ^a	4.29±1.02 ^a
	10.0	0.98±0.20 ^a	28.21±5.26 ^a	3.24±0.88 ^a	49.20±8.23 ^a	1.01±0.12 ^a

Values are expressed as mean±SEM (n=5). Significant at P < .05 when compared to negative control

3.3 Light Microscopy

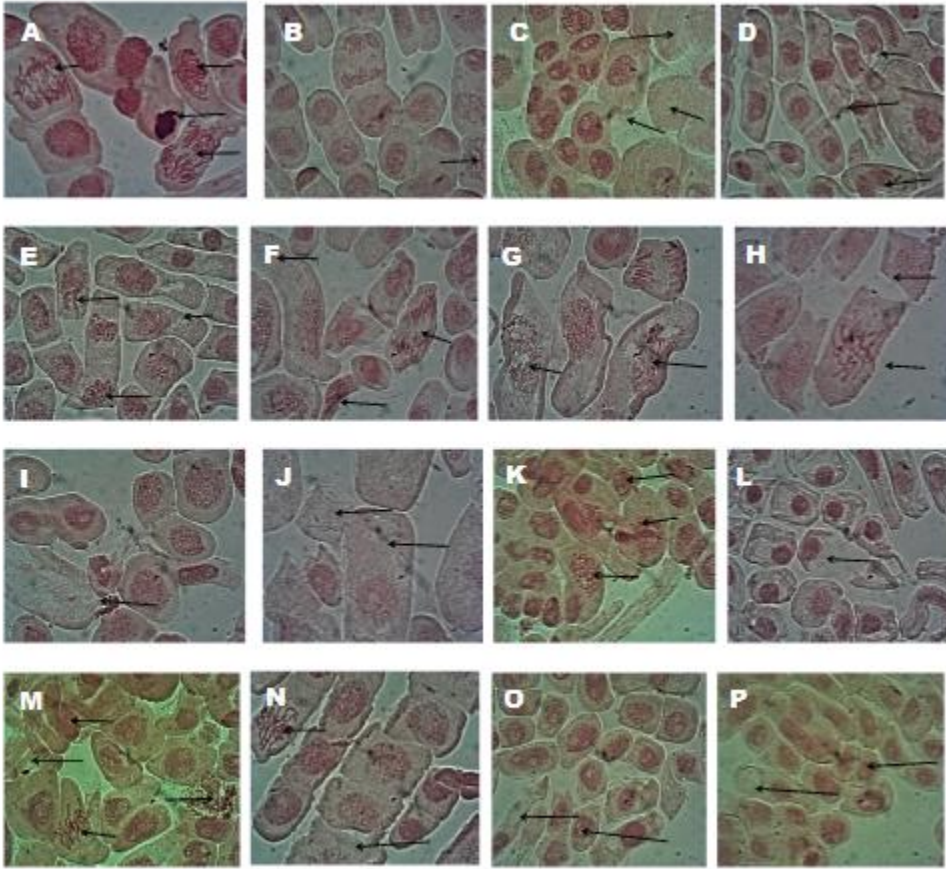


Fig.1. Photomicrograph showing the mitotic and chromosomal aberrations of *Allium cepa* root meristem cells after *Lasianthera africana*, *Heinsia crinata* and *Justicia insularis* leaf extracts treatment under light microscope X40 magnification. Arrows indicate (A) Chromosomal bridges (B) Sticky chromosomes (C) Ghost cells (D) Membrane and nuclear damage (E) Sticky telophase (F) Polar deviation (G) nucleus damage (H) Fragmentation (I) Nucleus damage (J) Apoptotic bodies (K) Sticky metaphase (L) Membrane damage (M) and (N) Laggard chromosomes (O) Micronucleus (P) binucleated cells

4. Discussion

In this study, toxic effects of leaf extracts of three vegetables; *L. africana*, *H. crinata* and *J. insularis* were evaluated by analyzing root growth and root morphology of *Allium cepa*. Varying concentrations of the extracts were observed to cause inhibition of root growth and these were statistically significant when compared to control group. In addition, the leaf extracts caused colouration of the root tips of *A. cepa* depending on the concentration. "This colouration ranged from yellowish, light brown to dark brown coloration of the roots. Cyto- and genotoxicity were estimated by observing 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". [57] Significant inhibition in the onion roots treated with the *L. africana* leaf extract had mitotic indices of 28.20%, 12.80% and 11.0% for 2.5, 5.0 and 10.0 mg/mL respectively, while *Heinsia crinata* leaf extract had mitotic indices of 32.60%, 19.00% and 14.00% respectively for 2.5, 5.0 and 10.0 mg/mL. Mitotic indices for 2.5, 5.0 and 10.0 mg/mL of *J. insularis* were 30.62%, 15.40% and 10.48% respectively compared to the negative control (Table 2). The inhibition of root growth was found to be dependent on decrease of mitotic Index. The mitotic index value below 22% when compared to negative control portrays a serious toxic effect on the

organism [35], while a value below 50% usually indicates sublethal effects [36] and is called cytotoxic limit value [37]. "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" [38]. "Reduction in the mitotic activity could be due to inhibition of DNA synthesis or a blocking in the G2 phase of the cell cycle, preventing the cell from entering mitosis" [39]. "Mitodepressive effects of some herbal extracts, including the ability to block the synthesis of DNA and nucleus proteins, were reported earlier" [40, 41]. "Several other herbal extracts have been reported to inhibit mitosis" [4, 42, 43]. "The decreased mitotic indices in *A. cepa* roots treated with *L. africana*, *H. crinata* and *J. insularis* leaf extracts were probably due to either disturbances in the cell cycle or chromatin dysfunction induced by 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* and it can prevent DNA synthesis and the reduction in number of the dividing cells in roots produced by 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 three extracts. Metaphases with sticky chromosome, loses their normal appearance, and they are seen with a sticky "surface," causing chromosome agglomeration" [44]. "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" [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]. "Fragments were observed in this study especially in the *Heinsia crinata* leaf extract treated groups. This extract used 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 *Lasianthera africana* leaf extract treated groups. Frequencies of total chromosome aberrations increased significantly following exposure to the extracts which indicate clastogenic activity (Table 3). These were more frequent aberrations in the groups treated with the *L. africana* leaf extract. The leaf 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 2.5 and 10.0 mg/mL of *J. insularis* 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 except *H. crinata* treated groups. The frequency of cells with micronuclei 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" [48, 49]. Previous studies have suggested MNC-induced effect of various plant extracts such as *Lavandula stoechas* and *Ecballium elaterium* [4, 50], *Azadirachta indica* [51], *Psychotriaspecies* [43].

In this study, membrane damage cells were observed in groups treated with various concentrations of the leaf extracts 2.5, 5.0 and 10.0 mg/mL but mostly in the *J. insularis* (10mg/mL) treated group. "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 leaf extracts. Multinucleated and binucleated cells were observed in extracts-treated groups. This is due to the prevention of cytokinesis or cell plate formation. Microtubules have been associated with cell plate formation and the extracts the process, leading to inhibition of cytokinesis. Ghost cell is a dead cell in which the outline remains visible, but whose nucleus and cytoplasmic structures are not stainable" [50]. "Some ghost cells were observed in various frequencies in this study in all the three extracts but mostly in *L. africana* leaf extract (10 mg/mL) treated groups (Figure 1). 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 (5 mg/mL and 10 mg/mL) of the extracts were found 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" [57].

The results of this study show that the extracts of *L. africana*, *H. crinata* and *J. insularis* 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.

The leaf extract of *H. crinata* is reported to contain two triterpenoid saponins [29] with high flavonoid and phenolic contents such as quercetin, ellagic, chlorogenic and caffeic acids found to be abundant in the leaves [52]. "Moreso, two iridoids ; lamalbid 6, 7, 8- triacetate and aglycone lamiridosin 6, 7, 8-triacetate have also been isolated from the stem bark [30]. Phytochemical screening of the leaf extract of *J. insularis* revealed the presence of saponins, alkaloids, tannins, flavonoids, anthraquinones and cardiac glycosides" [15, 53]. "Clerodane diterpenoids; 16(α/β)-hydroxy-cleroda-3,13 (14)Z-dien-15,16-olide and 2, 16-oxo-cleroda-3,13(14)E-dien-15-oic acid have also been isolated and characterised from the

leaf extract” [54]. According to Adegoke and Adebayo (2009), “phytochemical analysis of *Lasianthera africana* revealed the presence of alkaloids, saponins, tannins, flavonoids, cardiac glycosides, anthraquinones, and cyanogenetic glycosides in varying concentration” [55]. “Polyphenolic compounds such as quercetin, quercetin 3-methyl ether, luteolin, rutin, quercetin-3-O-rutinoside (rutin), α -L-rhamnopyranosyl-(1–6)- β -D-glucopyranose) have been isolated and identified from the leaves of *L. Africana* [31].

The high phenolic and flavonoid contents of the leaf extracts as well as triterpenoids may have been responsible for the observed effects in this study. Flavonoids such as quercetin have been reported to demonstrate mutagenic and genotoxic potentials in various studies [56] and this compound is present in the leaf extracts of these vegetables thereby accounting for the observed activities. The high phenols and flavonoids contents in the leaf extract must have contributed to the observed cytotoxic and genotoxic activities in this study.

5. CONCLUSION

This study revealed that the leaf extract of *Lasianthera africana*, *Heinsia africana* and *Justicia insularis* possess cytotoxic and genotoxic effects, as seen in the effects elicited by all test concentrations of the three plant 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 *Lasianthera africana*, *Heinsia crinata* and *Justicia insularis*.

REFERENCES

1. Higashimoto M, Purintrapiban J, Kataoka K, Kinouchi T, Vinitketkumnuen U, Akimoto S., Matsumoto H., & Ohnishi Y. (). Mutagenicity and antimutagenicity of extracts of three spices and a medicinal plant in Thailand. *Mutation Research*. 1993; 303(3): 135–142.
2. Schimmer O, Kruger A, Paulini H, Haefele F. An evaluation of 55 commercial plant extracts in the Ames mutagenicity test. *Pharmazie*. 1994; 49(6): 448–451.
3. Kassie F, Parzefall W, Musk S. 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*. 1996; 102(1): 1–16.
4. Çelik TA, Aslantürk ÖS. Cytotoxic and genotoxic effects of *Lavandula stoechas* aqueous extracts. *Biologia*. 2007; 62: 292–296
5. Ames BN. Food constituents as a source of mutagens, carcinogens, and anticarcinogens. *Progress in Clinical and Biological Research*. 1986; .206: 3–32.
6. Fernandes De Sá Ferreira IC, Ferrão Vargas VM. Mutagenicity of medicinal plant extracts in Salmonella/microsome assay. *Phytotherapy research: PTR*. 1999; 13(5): 397–400.
7. Wynder EL, Hall NEL. Polansky M. Epidemiology of coffee and pancreatic cancer. *Cancer Research*. 1983; 43(8): 3900–3906.
8. Nagao M, Wakabayashi K, Fujita Y, Tahira T, Ochiai M, Sugimura T. Mutagenic compounds in soy sauce, Chinese cabbage, coffee and herbal teas. *Progress in Clinical and Biological Research*. 1986; 206: 55–62.
9. Nguyen T, Fluss L, Madej R, Ginther C, Leighton T. The distribution of mutagenic activity in red, rose and white wines. *Mutation Research*. 1989; 223(2): 205–212.
10. Brito MT, Martinez A, Cadavid NFC. Mutagenic activity in regional foods and beverages from the Venezuelan Andean region. *Mutation Research*. 1990; 243(2):115– 120.
11. Okokon JE, Antia BS, Umoh EE. Antiulcerogenic activity of ethanolic leaf extract of *Lasianthera africana*. *African Journal of Traditional, Complementary, and Alternative medicines*. 2009a; 6(2): 150–154.
12. Okokon JE, Umoh EE, Jackson CL, Etim EI. Antiplasmodial and antidiabetic activities of *Heinsia crinata*. *Journal of Medicinal Food*. 2009b; 12(1): 231 - 236.
13. Telefo PB, Tagne SR, Koono OE, Yemele DM, Tchouanguép FM. Effect of the aqueous extract of *Justicia insularis* T. Anders (Acanthaceae) on ovarian folliculogenesis and fertility of female rats. *African journal of traditional, complementary, and alternative medicines*. 2011; 9(2): 197–203.
14. Ajibesin KK, Ekpo BA, Bala DN, Essien EE, Adesanya SA. Ethnobotanical survey of Akwa Ibom State of Nigeria. *Journal of Ethnopharmacology*. 2008; 115: 387 – 408.

15. Telefo PB, Moundipa PF, Tchouanguép FM. Inductive effect of the leaf mixture extract of *Aloe buettneri*, *Justicia insularis*, *Dicliptera verticillata* and *Hibiscus macranthus* on in vitro production of estradiol. *Journal of Ethnopharmacology*. 2004; 91: 225–230.
16. Adeyemi OT, Babatunde O. Chemical composition and anti-oxidant capacity of the leaf extract of *Justicia insularis*. *International Journal of Physical Sciences*. 2014; 9: 454–458.
17. Okokon JE, Antia BS, Essiet GA. Evaluation of in vivo antiplasmodial activity of ethanolic leaf extract of *Lasianthera africana*. *Research. Journal of Pharmacology*. 2007; 1(2): 30-33.
18. Andy IE, Eja ME, Mbotto CI. An evaluation of the antimicrobial potency of *Lasianthera africana* (Beauv) and *Heinsia crinata* (G. Taylor) on *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Candida albicans*. *Malaysian Journal Microbiology*. 2008; 4(1): 25 – 29.
19. Morah FN, Ashipu LB. Chemical composition and antimicrobial activity of essential oil from *Heinsia crinata* leaf. *American Journal of Essential Oils and Natural Products* 2017; 5(2): 23-28.
20. Okokon JE, Etebong EO, Umoh EE, Essien GA. Antiulcerogenic activity of ethanolic leaf extract of *Heinsia crinata*. *Journal of Pharmacy and Bioresources*. 2010; 7(2): 114-119.
21. Okokon JE, Davies K, Edem UA, Nyong EE, Basse AL. Anticonvulsant activity of ethanol leaf extract of *Heinsia crinata*. *Nigerian Journal of Pharmaceutical and Applied Sciences Research*. 2021; 10(1): 20-25.
22. Ebong PE, Igile GO, Mgbeje BIA, Iwara IA, Odongo AE, Onofiok UL, Oso EA. Hypoglycemic, hepatoprotective and nephroprotective effects of methanolic leaf extract of *Heinsia crinita* (rubiaceae) in alloxan-induced diabetic albino wistar rats. *IOSR Journal of Pharmacy*. 2014; 4(1); 37-43.
23. Ekanem NG, Mbagwu HOC, Harry GI. Phytochemical screening and hypoglycaemic activity of *Lasianthera africana* Beauv. (Aquifoliales: Stemonuraceae) leaf extract on diabetic rats. *Brazilian Journal of Biological Sciences*. 2016; 3(6): 293–298.
24. Inyang UE, Ani JC, Ebenso IE. Hypoglycaemic and hypolipidemic effects of *Lasianthera africana* leaf powder in alloxan-induced diabetic rats. *Nigerian Food Journal*. 2016; 34(1): 1-8.
25. Andrew UE, Okokon JE, Idiong OJ. Antiinflammatory and analgesic activities of *Heinsia crinata*. *Molecular and Clinical Pharmacology* 2012; 3(1): 30-39.
26. Okokon JE, Enin GN, Udofia OE, Amazu LU. Antinociceptive and antipyretic activities of leaf extract of *Lasianthera africana*. *Nigerian Journal of Pharmaceutical and Applied Sciences Research*. 2013a; 2(1): 17 - 24.
27. Okokon JE, Dar A, Choudhary MI. Chemical composition of *Lasianthera africana* and their immunomodulatory and antileishmanial activities. *Journal of Natural Products*. 2013b; 6: 27- 32.
28. Wood J, Yasmin-Karim S, Moreau M, Kumar R, Akwanwi J, Derek A, Atoneche F, Kress J, Ngwa AW. Characterization of Isolated Extracts from *Justicia* Plant Leaves used as Remedy for Anemia. *Molecules* (Basel, Switzerland). 2020; 25(3): 534.
29. Babady-Billa J, Chantal W, Suzane T, Amuri K, Georges H. Two triterpenoid saponins from *Heinsia crinata*. *Phytochemistry*. 1994; 36(6): 1489–1492.
30. Tshisekedi Tshibangu P, Mutwale Kapepula P, Kabongo Kapinga MJ, Tujibikila Mukuta A, Kalenda DT, Tchinda AT, Mouithys-Mickalad AA, Jansen O, Cieckiewicz E, Tits M, Angenot L, Frédéric M. Antiplasmodial activity of *Heinsia crinita* (Rubiaceae) and identification of new iridoids. *Journal of Ethnopharmacology*. 2017; 196: 261–266.
31. Ekpo DE, Joshua PE, Ogidigo JO, Nwodo O. High resolution UPLC-PDA-QTOF-ESI-MS/MS analysis of the flavonoid-rich fraction of *Lasianthera africana* leaves, and in vivo evaluation of its renal and cardiac function effects. *Heliyon*. 2020; 6(7): e04154.
32. Corrêa GM, Alcantara AFD. Chemical constituents and biological activities of species of *Justicia*—A review. *Revista Brasileira de Farmacognosia*. 2012; 22: 220–238.
33. Okokon JE, Ochigbo EB, Johnny II, Anagboso MO, Ebong NO. Genotoxic and Cytotoxicity Activities of Leaf Extract of *Setaria megaphylla*. *Asian Journal of Biochemistry, Genetics and Molecular Biology*. 2023; 15(3): 56-64
34. Bakare AA, Mosuro AA, Osibanjo O. Effect of Simulated Leachate on Chromosomes and mitosis in roots of *Allium cepa* L. *Journal of Environmental Biology*. 2000; 21: 263–271.
35. Antosiewicz D. Analysis of the cell cycle in the root meristem of *Allium cepa* under the influence of ledakrin. *Folia histochemica et cytobiologica*. 1990; 28(1-2): 79–95.
36. Panda BB, Sahu UK. Induction of abnormal spindle function and cytokinesis inhibition in mitotic cells of *Allium cepa* by the organophosphorus insecticide fensulfothion. *Cytobios*. 1985; 42(167-168): 147–155.
37. Sharma CBSR. Plant meristems as monitors of genetic toxicity of environmental chemicals. *Current Science*. 1983; 52: 1000–1002.
38. Rojas E, Herrera LA, Sordo M. Mitotic index and cell proliferation kinetics for identification of antineoplastic activity. *Anti-Cancer Drugs*. 1993; 4(6): 637–640.

39. Sudhaka R, Ninge Gowda KN, Venu G. Mitotic abnormalities induced by silk dyeing industry effluents in the cells of *Allium cepa*,” *Cytologia*. 2001; 66(3): 235–239.
40. Mercykutty VC, Stephen, J. Adriamycin induced genetic toxicity as demonstrated by *Allium cepa* test. *Cytologia*. 1980; 45(4): 769-777.
41. Schulze E, Kirschner M. Microtubule dynamics in interphase cells. *Journal of Cell Biology*. 1986; 102(3): 1020–1031
42. Çelik TA, Aslantürk ÖS. Anti-mitotic and antigenotoxic effects of *Plantago lanceolata* aqueous extract on *Allium cepa* root tip meristem cells, *Biologia*. 2006; 61(6): 693–697.
43. Akinboro A, Bakare AA. Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants. *Journal of Ethnopharmacology*. 2007; 112(3): 470–475.
44. Babich H, Segall MA, Fox KD. The *Allium* test— a simple, eukaryote genotoxicity assay. *American Biology Teacher*, 1997; 59(9): 580–583.
45. Gömürçen AN. Cytological effect of the potassium metabisulphite and potassium nitrate food preservative on root tips of *Allium cepa* L. *Cytologia*. 2005; 70(2): 119–128.
46. Swierenga SHH, Heddle JA, Sigal EA. 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*. 1991; 246(2): 301–322.
47. Sharma A, Sen S. *Chromosome Botany*. Enfield. 2002; p. 45.
48. Albertini RJ, Anderson D, Douglas GR. IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. *Mutation Research*. 2000; 463(2):111–172.
49. Krishna G, Hayashi M. In vivo rodent micronucleus assay: protocol, conduct and data interpretation. *Mutation Research*. 2000; 455(1-2): 155-166.
50. Çelik TA, Aslantürk ÖS. Investigation of cytotoxic and genotoxic effects of *Ecballium elaterium* juice based on *Allium* test. *Methods and findings in experimental and clinical pharmacology*. 2009; 31(9): 591–596.
51. Soliman MI. Genotoxicity testing of neem plant (*Azadirachta indica* A. Juss.) using the *Allium cepa* chromosome aberration assay. *Journal of Biological Sciences*. 2001; 1(11): 1021–1027.
52. Ganiyu O, Nwanna EE, Oyeleye SI, Olasehinde TA, Ogunsuyi OB, Bolington AA. In vitro neuroprotective potentials of aqueous and methanol extracts from *Heinsia crinita*. *Food Science and Human Wellness*. 2016; 5(2), 95-102.
53. Oyomah GO, Odoemena CS, Ogede RO, Bala D. Analgesic and anti-inflammatory activities of the ethanol leaf extract of *Justicia insularis* (T. Anders) on albino rats. *Journal of Advanced Research*. 2019; 1(2): 1-6.
54. Fadayomi IE, Johnson-Ajinwo OR, Pires E, McCullagh J, Claridge TDW, Forsyth NR, Li WW. Clerodane diterpenoids from an edible plant *Justicia insularis*: discovery, cytotoxicity, and apoptosis induction in human ovarian cancer cells. *Molecules*. 2021; 26: 5933.
55. Adegoke AA, Adebayo-Tayo BC. Antibacterial activity and phytochemical analysis of leaf extracts of *Lasianthera africanum*. *African Journal of Biotechnology*. 2009; 8(1): 077-080.
56. Ping KY, Shohaimi S, Sasidharan S, Yusuf UK. Genotoxicity of Selected Chinese Medicinal Plants, *Elephantopus scaber*, *Glycyrrhiza uralensis* and *Salvia miltiorrhiza* on *Allium cepa* Assay. *Annals of Pharmacology and Pharmaceutics*. 2017; 2(13): 1070.
57. Aşkin Çelik T, Aslantürk ÖS. Evaluation of cytotoxicity and genotoxicity of *Inula viscosa* leaf extracts with *Allium* test. *Journal of biomedicine and biotechnology*. 2010;2010.