

## GENOTOXIC AND CYTOTOXIC ACTIVITIES OF leaf EXTRACT OF *Stachytarpheta cayennensis* (Rich ).Vahl

### Abstract

The many pharmacological potentials of *Stachytarpheta cayennensis* (L.C. Rich) Vahl, (**verbenaceace**), commonly called blue snakeweed and its use in traditional medicine in Nigeria such as in the treatment of hypertension, diabetes, mental illness and infections, could position it as a medicinal plant of abuse, necessitating the need to ascertain its safety. This study therefore investigated the genotoxic and cytotoxic activity of ethanolic leaf extract of *Stachytarpheta cayennensis* using the *Allium cepa* test. The effect of the leaf extract on the root meristem cells of *Allium cepa* was investigated using onion bulbs exposed to 2.5 mg/mL, 5 mg/mL, and 10 mg/mL concentrations of the extract 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 extract when compared with the negative control group. All the tested concentrations of the extract were observed to exert cytotoxic effects on cell division in *A. cepa*. The extract-induced chromosomal aberrations and micronuclei (MNC) formations in *A. cepa* root tip cells were significant ( $p < 0.05$ ) when compared with control group. The leaf extract treatment further induced cell death, ghost cells, cells membrane damage, and binucleated cells. These results suggest that the leaf extract of *Stachytarpheta cayennensis* possess cytotoxic and genotoxic effects on *A. cepa*.

**Keywords:** *Stachytarpheta cayennensis* , genotoxic , cytotoxic

### INTRODUCTION

*Stachytarpheta cayennensis* (L.C. Rich) Vahl (**verbenaceace**) is a weedy (and sometimes perennial) herbaceous plant commonly called Brazilian tea. Two common very similar species of *Stachytarpheta cayennensis* grow in the tropics and are used interchangeably (and share the same common names) in the herbal medicine systems of many countries, *Stachytarpheta cayennensis* and *Stachytarpheta jamaicensis*. Ethnobotanically, *Stachytarpheta cayennensis* is used to treat various ailments such as inflammation, pain, fever, hepatic and renal disorder, helminthiasis, constipation, hypertension, stress and diabetes (**Burkhill, 1996**; Weniger, 1988; Rodriguez and Castro, 1996; Cano and Volpato, 2004). *S. cayennensis* is employed in traditional medicine for the management of mental illness and as an anti-inflammatory, antimalarial, analgesic, antipyretic, hepatoprotective, laxative agent, and in the treatment of gastric disorders (Sideney *et al.*, 2015). The plant is use in parts of southern Nigeria and Peru (Kvist *et al.*, 2006), for the treatment of malaria. Phytochemical studies of the plant revealed that it contains alkaloids (Alice *et al.*,

1991), Ipolamide, beta hydroxyipolamide and verbascoside (Kooiman, 1975; Schapoval *et al.*, 1998), steroids, triterpenes and irridoids (Futuro *et al.*, 1997). It is also considered to be most rich in iridoids glycosides, mainly ipolamiide, lamiide; phenylethanoid glycosides such as, jinoside-D, martinose (martynoside), acetoside, iso-acetoside, leucosceptoside-A. Arylpropanoid glycosides found are verbascoside and isoverbascoside (Sideney *et al.*, 2015). *Stachytarpheta cayennensis* has been reported to have antiinflammatory, antinociceptive, anti ulcerogenic (Schapoval *et al.*, 1998; Veal *et al.*, 1997; Penido *et al.*, 2006), antidiarrheal (Almeida *et al.*, 1995) as well as sedative (Akanmu *et al.*, 2005) and hypotensive (Idu *et al.*, 2006) properties. Other biological activities such as Gastric acid secretion and antiulcer (Mesia-Vela *et al.*, 2004). immunomodulatory activity (Okoye *et al.*, 2014), antimalarial (Okokon *et al.*, 2008; Okoye *et al.*, 2014), antimicrobial and antispasmodic (Okoye *et al.*, 2010), antipsychotic (Olayiwola and Ibikunle, 2014), antifungal (Mekam, Pascal *et al.*, 2012), antioxidant (Onofre *et al.*, 2015), antidiabetic (Adebalo *et al.*, 2007) and antiasthmatic (Guimarães *et al.*, 2023) activities have been reported. In many resource limited countries like Nigeria, the use of plant parts for the treatment of diseases and overall health promotion and wellbeing has become an integral part of the healthcare system. This is for reasons of availability, affordability, acceptability and purportedly safety. This acclaimed safety has not been scientifically validated to a great extent for most plant drugs. This has given birth to a new awakening in the study of toxic potentials of many plant drugs in order to provide a guide to their use. Hence this study aims to assess the genotoxic and cytotoxic activity of *Stachytarpheta cayennensis* using *Allium cepa* test.

## **Materials and Methods**

### **Plants collection**

The plant material *Stachytarpheta cayennensis* (leaves) were collected in Uruan area, in Akwa Ibom State, Nigeria in April 2024. The plant was identified and authenticated by a taxonomist in Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria.

### **Extraction**

The leaves were washed (to remove contaminants) and shade-dried for two weeks.

The dried plants' materials were further chopped into small pieces and reduced to powder using electric grinder. The powdered leaves material (1.5 kg) was macerated

for 72 h in 50% ethanol. This was thereafter filtered and the liquid filtrate was concentrated and evaporated to dryness in *vacuo* 40°C using a rotary evaporator (BuchiLab, Switzerland). The extract was stored in a refrigerator at -4°C, until used for the proposed experiments.

### ***Allium cepa* test.**

This was carried out according to the method of Grant (1994) and Ikechukwu *et al.*, (2024). Small onions bulbs, *A. cepa*, were procured from Itam market, Itu LGA, Akwa Ibom State, Nigeria. The bulbs were first authenticated by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo. The bulbs were then processed for the study by scarifying the bulbs and bottom base without destroying the root primordia using a small sharp knife. Distilled water (200 mL) was used to reconstitute the extract (20 g) which was thereafter diluted to different concentrations of the extract 2.5 mg/mL, 5 mg/mL and 10 mg/mL respectively from the stock solution. Test concentrations of the leaf extract at 2.5 mg/mL, 5 mg/mL, and 10 mg/mL concentrations were filled in 50 mL beakers and arranged in a series of 5 per test 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. 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. 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 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 (Bakare *et al.*, 2000; Magnus *et al.*, 2024). 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 in evaluating cytotoxicity and genotoxicity of the leaf extract (i) the mitotic index (MI) (ii) chromatin aberrations (stickiness, bridges,

breaks and polar deviation) and micronuclei (MNC) were scored per 500 cells (Bakare *et al.*, 2000; Magnus *et al.*, 2024).

### ***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 effect of *Starchytarpheta cayenensis* leaf extract on levels of the physicochemical parameters (root number and root length) are presented in Table 1. This result shows that all tested concentrations **of** *Starchytarpheta cayenensis* leaf extract caused significant inhibition in the growth of roots in comparison to negative and positive control groups. The inhibition of root number and root length was greater with increasing concentrations of the leaf extract. The average root length in negative and positive control (methotrexate) groups were  $4.56 \pm 0.19$  and  $0.10 \pm 0.01$  cm respectively. However, average root length in 10 mg/mL treatment group was decreased significantly compared to that of the negative control;  $0.24 \pm 0.05$  cm for *S. cayenensis* (Table 1). Average root lengths in treatment groups were decreased depending on concentration, significantly ( $p < 0.05$ ) when compared to negative control. The root morphology was almost normal during the negative control treatment, but at 2.5 mg/mL of *S. cayenensis* leaf extract, the roots appeared slightly yellow and at 5 and 10 mg/mL of *S. cayenensis* leaf extract, the roots had brownish tips. (Table 1).

### **Cytogenetic Analysis.**

Table 2 shows the effects of *S. cayenensis* leaf extract on cytogenetic parameters of *Allium cepa* roots. Cytogenetic analysis performed showed that the fruit extract caused concentration-dependent and significant ( $p < 0.05$ ) decreases in the mitotic index when compared to that of negative control. The leaf extract of *S. cayenensis* at 10 mg/mL had mitotic index of  $6.40 \pm 2.88$  as compared to  $69.90 \pm 5.64$  recorded in the negative control group (Table 2).

Cytogenetic alterations caused by the extract are shown in Table 3. Chromosome and cytological alterations were observed in negative control, methotrexate, *S. cayenensis* leaf extract-treated groups as depicted in Table 3. Analysis of chromosome aberrations observed showed that there were bridges of chromosomes and nuclear damage detected in the different concentration treatments (Table 3) (Figure 1(a)). These were significant ( $p < 0.05$ ) when compared to negative control group. No fragments or clastogenic breaks of chromosomes was observed in all concentrations of leaf extract except in methotrexate treated group (Table 3; Figure 1 (B,C)). Sticky metaphase were also observed (Figures 1(E)) in the extract-treated groups but were more frequent in the group treated with the highest concentration of the extract (10 mg/mL). It was generally observed that these abnormalities increased with increasing concentrations of the extract. A concentration-dependent and statistically significant ( $p < 0.05$ ) increase in total aberrant cells (aberrant cells include chromosome breaks, stickiness and polar deviation) as compared with the negative control (Table 3) was observed. However, the highest value of aberrant cells was observed in methotrexate-treated group (positive control) (Table 3). Genotoxic activities of the extract 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 2.5 mg/mL of *S.*

*cayenensis* had high numbers of cells with micronuclei in the test compared to negative control, which were statistically significant ( $p<0.05$ ) (Figure 1(D)). In addition, cells with membrane damage (Figure 1(B, E)), binucleated cells (Figure 1(D,E,F)), and nucleus damage (Figures 1(A, B)) were found in various frequencies. Also, dead and apoptotic cells (Figure 1(A)) were detected in the group treated with the leaf extract.

Table 1: Cytotoxicity of *Stachytarpheta cayenensis* 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	21.25 $\pm$ 1.54	4.56 $\pm$ 0.19
Methotrexate	0.1	2.10 $\pm$ 0.02 <sup>a</sup>	0.10 $\pm$ 0.01 <sup>a</sup>
<i>Stachytarpheta cayenensis</i>	2.5	8.66 $\pm$ 2.33 <sup>a</sup>	0.66 $\pm$ 0.06 <sup>a</sup>
	5.0	7.66 $\pm$ 1.33 <sup>a</sup>	0.46 $\pm$ 0.06 <sup>a</sup>
	10.0	4.25 $\pm$ 1.66 <sup>a</sup>	0.24 $\pm$ 0.05 <sup>a</sup>

Values are expressed as mean  $\pm$ 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 (%) $\pm$ S.E
Negative control	Tap water	500	348	69.60 $\pm$ 5.64
Methotrexate	0.1	500	15	3.00 $\pm$ 0.68 <sup>a</sup>
<i>Stachytarpheta cayenensis</i>	2.5	500	61	12.20 $\pm$ 3.76 <sup>a</sup>
	5.0	500	42	8.40 $\pm$ 2.68 <sup>a</sup>
	10.0	500	32	6.40 $\pm$ 2.88 <sup>a</sup>

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

Table-3: Chromosomal and mitotic aberrations in the root meristematic cells of *Allium cepa* after treatment with *Stachytarpheta cayenensis* leaf extract

**A** ←      **B** →      ← **C**

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	-	0.05±0.06	-	2.00±0.28	-
Methotrexate	0.10	2.34±1.23 <sup>a</sup>	21.34±5.38 <sup>a</sup>	10.55±2.28 <sup>a</sup>	45.13±4.22 <sup>a</sup>	2.28±0.86 <sup>a</sup>
<i>Stachytarpheta cayenensis</i>	2.5	-	2.14±0.34 <sup>a</sup>	-	28.98±4.16 <sup>a</sup>	4.18±0.22 <sup>a</sup>
	5.0	-	5.54±2.96 <sup>a</sup>	-	34.18±5.35 <sup>a</sup>	2.49±1.98 <sup>a</sup>
	10.0	-	6.54±1.48 <sup>a</sup>	-	51.45±5.26 <sup>a</sup>	-

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

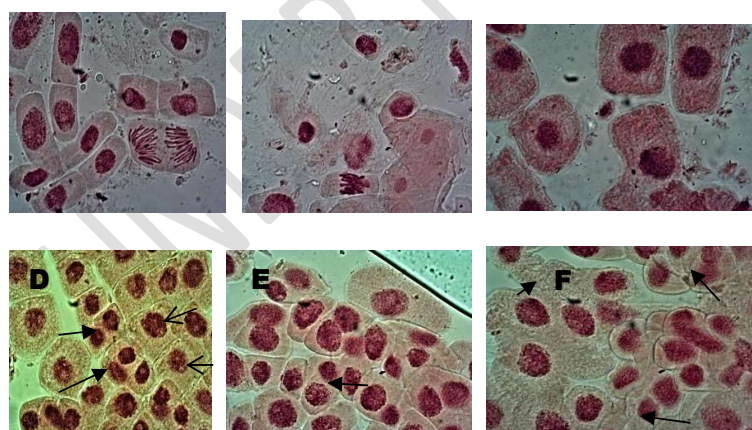


Figure 1: Photomicrograph showing the mitotic and chromosomal aberrations of *Allium cepa* root meristem cells after *Stachytarpheta cayenensis* leaf extract treatments under light microscope X40 magnification. Arrows indicate (A) Apoptotic cells and nuclear damage (B) fragmentation, membrane, cell wall and nuclear damage (C) fragmentation, membrane damage and cell wall damage (D) Binucleated and multinucleated cells (E) Binucleated cells, membrane damage and sticky metaphase (F) binucleated cells and nuclear damage



## Discussion

In this study, toxic effects of *S. cayennensis* leaf extract was evaluated by analyzing root growth and root morphology. Varying concentrations of the extract were observed to cause inhibition of root growth and these were statistically significant when compared to control group. In addition, the extract induced slightly yellow, slightly brown and brownish 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 (3.60%) was significantly decreased when compared to control. Significant inhibition in the onion roots treated with the *Stachytarpheta cayennensis* leaf extract (12.20%, 8.40% and 6.40% compared to the negative control) was observed (Table 2). The inhibition of root growth was found to be dependent on decrease of Mitotic Index. The decline of mitotic index below 22% in comparison to negative control can have lethal impact on the organism (Antonsie-Wiez, 1990), while a decrease below 50% usually has 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.*, 2001). 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 (Sudhakar *et al.*, 2001). Mitodepressive effects of some herbal extracts, including the ability to block the synthesis of DNA and nucleus proteins, were reported earlier (Mercykutty and Stephen, 1980; Schulze and Kirschner, 1986). Several other herbal extracts have been reported to inhibit mitosis (As *et al.*, 2007; As *et al.*, 2006; Akinboro and Bakare, 2007). The decreased mitotic index in *A. cepa* roots treated with *Stachytarpheta cayennensis* leaf

extract is probably due to either disturbances in the cell cycle or chromatin dysfunction induced by extracts-DNA interactions. The results herein suggest that the tested extract concentrations have inhibitory, mito-depressive effects on root growth and cell division of *A. cepa* 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 extract. The observation of sticky metaphase reinforces the hypothesis of the toxic effect of the extract. Metaphases with sticky chromosome, loses their normal appearance, and they are seen 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 (G’om’urgen, 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 in all the extract concentrations- treated groups. The extract was found to not only interfere with the cell cycle, but also affect chromatin organization or DNA replication, causing chromosome breaks. Frequencies of total chromosome aberrations increased significantly following exposure to the extract which indicate clastogenic activity (Table 3). The extract significantly induced the formation of MNC in *A. cepa* root cells at 2.5–10 mg/mL concentrations. Frequencies of MNC was found to increased in the groups treated with 2.5 mg/mL of the stem extract. However, MNC frequency decreased in *A. cepa* roots treated at the highest concentration of the

extract (10 mg/mL), due to high cytotoxicity. 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 (Albertini *et al.*, 2000; Krishna and Hayashi, 2000). Previous studies have suggested MNC-induced effect of various plant extracts such as *Hippocratea africana* (Johnny *et al.*, 2023), *Setaria megaphylla* (Okokon *et al.*, 2023), *Heinsia crinata*, *Lasianthera africana* and *Justicia insularis* (Ikechukwu *et al.*, 2024), *Solanum anomalum* fruit (Okopide *et al.*, 2024), *Croton zambesicus* (Osigwe *et al.*, 2025).

In this study, membrane damaged cells were observed in all the treated groups. These results indicated the potential of the extract to exert cytotoxic effect over certain concentrations such as cause membrane damage. Multinucleated and binucleated cells have been observed in extract treated groups. This is due to the prevention of cytokinesis or cell plate formation. Microtubules have been implicated in cell plate formation and the extracts 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 especially in 10 mg/mL treated groups (Figure 2). This could have resulted from the activities of the phytochemical constituents of the extract leading to nucleus damage and prevention of cytoplasmic structures, thus resulting in ghost cells. In addition, the extract also induced DNA damage and cell death and/or apoptosis in various frequencies in this study. 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.

## Conclusion

The results of this study show that the leaf extract of *S. cayenensis* 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*, suggesting cytotoxic and genotoxic activities of the extract.

Therefore, proper use of these plants in ethnomedicine is recommended and high doses should be avoided as it can cause cytotoxic and/or genotoxic effects.

## DISCLAIMER ARTIFICIAL INTELLIGENCE (AI)

Authors hereby declare that no AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript

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