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*Original Research Article*

# Cytotoxicity Evaluation and the Free Radical Scavenging Activity of the Root Extracts of *Calotropis procera*

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ABSTRACT

Health consequences following the unethical consumption of herbal products call for serious safety concern. This study investigated the cytotoxic and antioxidant activity of ethylacetate root extract (ETAE) and hexane root extract (HEXE) of *Calotropis procera* by applying the methods of *Allium cepa* and spectrophotometry respectively. Total phenolics content was concentrated in the root extracts, followed by total alkaloid and total flavonoids content. However, these phytochemicals were concentrated in ETAE compared to the HEXE. The results of the 50% inhibitory concentration obtained from the ferric reducing power and 2,2-diphenyl-1-picrylhydrazyl, showed that ETAE had stronger anti-free radical activity compared to the HEXE. Furthermore, the ETAE reduced thiobarbituric acid reactive species considerably than the HEXE. Exposing the onion bulbs to the test solutions (ETAE, HEXE and formalin), degenerated cells and amitotic divisions were observed after 72 hours of exposure compared to the 24 hours and 48 hours exposures. The formalin solution showed cytotoxic potential (reduced rate of root growth and mitotic index) in comparison with the root extracts, however, ETAE's effect was lower than that shown by the HEXE. Also, the root extracts induced chromosomal abnormalities like sticky, vagrant, bridge, elongated and enlarged cells which may be attributed to the interaction between the plant's alkaloid content and the onion's chromosomal apparatus. In conclusion, the plant's high concentration of total phenolics content may have activated both the anti-free radical and modulatory activity.

***Keywords***: Antioxidant activity, Medicinal plant, Mitotic index, Chromosomal aberrations, Mitotic divisions, Phytochemicals.

## Introduction

Biological processes like autoxidation, oxido-reduction and lipid peroxidation reactions have resulted in the formation of unstable molecules called reactive species (or free radicals) – hydroxyl radicals, superoxide anions, lipid peroxyl radicals, 2,2-diphenyl-1-picrylhydrazyl radicals, are identified as the causative agents of many health abnormalities suffered by both animals and humans.[1,2] Nonetheless, the characterization of medicinal plants indicated that they are blessed with diverse range of phytochemical compounds with potent antioxidant activity, capable of mitigating the harmful effects of reactive species by increasing free radical's stability via electron donation or scavenging of excess free radicals from the system.[1,3]
The application of phytomedicinal species for both pharmaceutical and nutritional purposes in disease treatment started from ancient times and has increasingly become domesticated in developing nations, because they are always available, accessible, affordable, potent and safe compared to modern medicines. However, issues such as organ damage, cellular apoptosis and death have been reported arising from abuse of traditional medicines.

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Probing into the negative outcomes associated with herbal consumption, published articles attributed it to toxicity - intoxication of the system when exposed to substances above a clinically recommended concentration per time. Toxic compounds may elucidate their harmful effects by inhibiting key metabolic processes, resulting in health disorders, followed by outcomes such as behavioral perversion, organ disruption (or aberrations) and death.[4, 5]
Despite the therapeutic roles associated with the use of traditional medicines, the probability of it being toxic may be minimal. Nevertheless, the validation of the toxicological status of medicinal plants/drugs was necessary. In the past, bioassays like *Allium cepa*, Brine shrimp lethality, Ames test, cell lines were opaque but gradually gained general acceptance, as their results were comparable in animal and human systems. Studies have documented a positive correlation between toxicity and these assays as exemplified in the detection of the antitumor activity in organisms.[6,7] *Calotropis procera* is a succulent plant and a member of the *Asclepiadaceae* family. The plant is distributed in severely dry tropical regions and survives by means of its tap roots for water uptake and storage in tissues. Furthermore, it also contains thick cuticle and limited stomata cells that reduce transpiration rate.[8] The plant bears the following English names like rooster tree, auricular tree, Swallow-wort, rubber-bush, Giant milkweed, small crown flower, Sodom apple and Dead Sea apple while the major tribes in Nigeria, the Hausas, Yorubas and Igbos named it as Tumfafiya, Bom-Ubomu and Otosi respectively. Recent published articles have established ethno-pharmacological properties of the root extract of *C. procera* but not limited to the following, nephroprotective, hematoprotective, anti-diabetic and antioxidant properties.[9,10] Notably, bioactive compounds like rutin (quercetin-3-rutinoside), lupeol, ursolic acid, α and *β* Amyrin, Benzolisoleneolone, Calactinic acid, calotropin, Gofruside, oleanolic acid, procerursenyl



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acetate, terpenoid glycoside, Diterpene, methyl myristate among others have been isolated from the root extract of *C.procera*.[10,11] To the best of our knowledge, the cytotoxic record of the plant's root using *Allium cepa* is unavailable. Considering this research gap, this study invesrigated the cytotoxicity potential, antioxidant activity and phytochemical content of the root extracts of *C. procera* for the purposes of providing beneficial information to users and researchers.

## Materials and Methods

The 2,2-diphenyl-1-picrylhydrazyl and Quercetin were purchased from Aldrich-Sigma (Germany). Gallic acid and ascorbic acid were acquired from China while ethylacetate, methanol and n-hexane solvents were ordered from BDH (England). Other chemicals used were of analytical grades purchased from reputable companies.

*Authentication of the Plant Sample*
The root sample (*C. procera*) was harvested in April, 2022 from Nigeria Police Academy, Wudil and authenticated by a taxonomist (Dr Aminu Jabbi), Department of Biological Science. A reference sample was further documented by the University's herbarium (Catalogue number NPAH 111).

*Extraction of the Plant Sample*
Cold maceration extraction was used, after 21 days of air-drying and pulverization of the root sample. Equal weight (1500 g) of the powdered root was extracted with equal volume (5000 mL) of the different organic solvents (ethylacetate and n-hexane). The extraction lasted for 48 hours with intermittent stirring. They were carefully filtered and the filtrates concentrated via evaporation and semi-solid root extracts were recovered weighing 3.17 g (ETAE) and 2.43 g (HEXE) respectively. The above procedure represented the method outlined by Ihegboro *et al.*[12]

*Measurement of phytochemical content in the root extracts*
*Measurement of the Total Phenolic Content*
The total phenolic content was quantified using the method of Niama *et al*.[13] To 1.0 mL of the root extract (1.0 mg/mL), add 1.0 mL of FC (folin-Ciocalteu's phenol reagent) and allowed to stand for 5 mins. Then 1.0 mL of 7% $Na_2CO_3$ solution and distilled water (13 mL) were added. The entire content was kept in a dark place for 90 mins, after which absorbance was taken at 750 nm. A calibration curve of gallic acid was plotted from where the concentration of total phenolics was extrapolated and expressed as milligram of GAE per gram.

*Measurement of Total Flavonoid Content*
The reaction mixture which contained 0.6 mL of the root extract, 6.8 mL of 30% methanol, 0.3 mL of 0.5 M $NaNO_2$ and 0.3 mL of 10% $AlCl_3.6H_2O$ were mixed very well. After 6 mins, 2.0 mL of 1.0 M NaOH was added and then made up with 10 mL of distilled water. The whole content was mixed and absorbance measured against the reagent blank at 506 nm. From the quercetin calibration curve (0-1.0 mg/mL), total flavonoids content was determined and expressed as milligram of rutin equivalent per gram. This was the method described by Nimala *et al.*[14]

*Measurement of Total Alkaloid Content*
The gravimetric technique used by Nantongo *et al*.[15] was replicated. Briefly, 0.5 g of the root extract was introduced into 10 mL of 10% acetic acid in ethanol and allowed to stay for 3 hours. The filtrate obtained was concentrated to a quarter of the initial volume, after which concentrated $NH_4OH$ was added dropwise to form a precipitate and then filtered. The content of the filter paper was dried in an oven heated at $60^0$C for 20 mins. The total alkaloid content was then quantified.

*Free Radical Scavenging Ability of the root extracts*
*DPPH free Radical scavenging Assay*
From the stock solution (1.0 mg/mL), different concentrations of the extracts were prepared (0-100 µg/mL). After addition of 1.0 mL of 0.1 M DPPH solution, the mixture was incubated in the dark at room temperature for a period of 30 mins. A control solution was prepared consisting of 1.0 mL methanol and 1.0 mL DPPH solution. The absorbance was read at a wavelength of 517 nm using a spectrophotometer Ascorbic acid was used as the standard. The above method was obtained from Nithianan *et al.*[16] Furthermore, the 50% inhibitory concentration ($IC_{50}$) was measured which represented the concentration required to reduce 50% DPPH concentration.[17]

*Ferric Reducing Power Assay*
The various concentrations of the root extract (25-100 µg/mL) were prepared. In a test tube containing 2.0 mL of 0.2 M phosphate buffer and 2.0 mL potassium ferricyanide (10 mg/mL), a 2.0 mL aliquot of the root extract was then added and mixed. The mixture was incubated at 50°C for 20 mins and afterward centrifuged at 3000 rpm. 2.0 mL trichloroacetic acid (100 mg/L) was added to the supernatant and stayed for 10 mins, followed by addition of 2.0 mL of distilled water and then 0.1% (w/v) newly prepared ferric chloride (0.8 mL). The absorbance was read at 700 nm after incubation of the resultant mixture for 10 mins. A calibration curve of ferric sulphate at different concentrations was plotted where the FRAP values were extrapolated. This was the method of Ononamadu *et al.*[18]

*Measurement of Thiobarbituric acid reactive species (TBARS)*
A 1.0 mL of reaction assay consisted of 0.58 mL of 0.1 M phosphate buffer (pH = 7.4), 0.2 mL liver homogenate and 1.0 mL of 0.67% thiobarbituric acid (TBA) was heated in a water bath for a period of 20 mins and then cooled with ice block pellets before centrifugation at 2000 x g for 10 mins. A spectrophotometer was used to determine the concentration of TBARS formed in the sample in relation to the reagent blank at 535 nm. The result was expressed in terms of micro molar (µmol). The methodology described above was used by Niama *et al*.[13]

*Cytotoxicity evaluation of the root extracts.*
*Allium cepa Assay*
After 24 hours of exposing the onion bulbs (*Allium cepa*) to distilled water, five onions with good root system were selected for the assay. They were exposed to the test solutions (formalin, ethylacetate root extract and hexane root extract) for a period of 72 hours in a dark place at room temperature. The root tip growth lengths were then measured every 24 hours using a ruler. After experimentation, a 5−7 mm onion root tip length was excised and preserved in a fixation solution containing ethanol-glacial acetic acid (3:1). The fixed root tips were then hydrolyzed in 1 N hydrochloric acid at 60°C for 6 mins and afterward rinsed with distilled water. On a glass slide, the root tips were squashed and later stained with 2% aceto-orcein for 10 mins. A slide cover was used to cover the content to avoid air bubbles and sealed with fingernail polish. For each concentration (0.1, 0.5 and 1.0 mg/mL) and the control, ten slides were prepared and then examined microscopically and photomicrographs were taken with the aid of a camera. The root tip growth, chromosomal aberrations and mitotic index were determined. This method was adopted from Ihegboro *et al.*[19]

*Data Analyses*
The data were in triplicates and expressed in terms of Mean ± SD by using the SPSS software version 16. Following that, ANOVA and Tukey's post hoc tests were conducted with a confidence level of $p < 0.05$.

## Results and Discussion

*Phytochemical constituents of the root extracts of C. procera*
A glance at Figure 1, showed that the root extracts contained a significant concentration of total phenolics content (TPC), whereas total flavonoids content (TFC) was found to be lower compared to the total alkaloid content (TAC). Comparing between the root extracts, the ethylacetate root extract (ETAE) was richer in total phenolics, alkaloid and flavonoids contents than the hexane root extract (HEXE). In comparison with aqueous root extract of *C. procera*, the ETAE and


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HEXE contained substantial concentration of total phenolics and flavonoids content.[20]

*Free radical scavenging Activity of the root Extracts*

Figure 2 showed a positive correlation between free radical scavenging activity and concentration. From the viewpoint of the 50% inhibitory concentration ($IC_{50}$), the ETAE (20.76 ± 0.20[a] µg/mL), showed similar free radical scavenging activity as the ascorbic acid (ASCA) (21.09 ± 0.21[a] µg/mL), but significantly inhibited the reactive species (2,2-diphenyl-1-picrylhydrazyl free radicals) compared to the HEXE (43.95 ± 0.09[b] µg/mL). As shown in Figure 3, a linear progression was established between redox activity and concentration. The ASCA's reducing activity ($IC_{50}$ = 80. 33 ± 0.12[a] µg/mL) was significantly stonger compared to the root extracts. However, the redox activity of the ETAE ($IC_{50}$ = 120.24 ± 1.37[b] µg/mL) was significantly higher compared to that of the HEXE ($IC_{50}$ = 156.20 ± 0.24[c] µg/mL).

Finally, the ETAE reduced thiobarbituric acid reactive species (TBARS) considerably when juxtaposed with the HEXE as shown in Figure 4. The results showed that the ETAE's anti-free radical activity was stronger than that of HEXE. This may be attributed to the huge concentration of total phenolics content in the ETAE compared to the level present in the HEXE. The phenolic's antioxidant role may be performed via the following mechanisms – proton donation, redox activity (as reducing agents), metal chelation or singlet oxygen quenching.[21, 22, 25, 26] Furthermore, study carried out using *Uncaria gambir* extract agreed with our finding, that high phenolics content activated strong antioxidant activity.[3]

*Screening for the cytotoxic effect of the root extracts*

Over time, toxicological result produced by *Allium cepa* has been considered more accurate, reliable and efficient compared to bioassays like micronucleus test (MN), Ame's test and cell lines.[19,5,27] This was because, Allium cepa provides molecular insight about the effect of toxicants on the structure of the chromosome. Secondly, the result obtained was 82% accurate when compared with mouse carcinogenicity/cytogenetic studies, and finally, the result recorded was similar to that of the human lymphocytes and rat's bone marrow test.[28-30] Studies suggested that decreased root growth and mitotic index signified potential mitodepressive, inhibitory, cytotoxic, cellular apoptosis, ATP depletion, impaired nucleoprotein synthesis and inhibition of DNA synthesis.[28-30] Looking at Figures 5 and 6, the formalin solution (positive control) substantially inhibited the onion's root growth and decreased mitotic index (% MI) compared to the root extracts. However, a good meristematic root growth and mitotic index was observed in the control group (distilled water) compared to the root extracts. In comparison with the 24 hours and 48 hours exposures, the test solutions caused cellular degeneration and amitosis after 72 hours of exposure, while the control (distilled water) produced normal cells at different stages of mitotic divisions (Table 1).

Furthermore, chromosomal aberrations such as anaphase bridge, sticky anaphase (evidence of irreversible genotoxicity), vagrant, disintegrated nucleus, enlarged and elongated cells were induced by the root extracts as shown in Figure 7(b) and 7(c), compared to the control group that showed normal mitotic divisions as represented in Figure 7(a), while the formalin solution induced mainly sticky anaphase/sticky chromosomes. The various aberrations induced by the root extracts may be attributed to the interaction of the plant's alkaloid content with the onion's chromosomal tubulin, thereby leading to deformity in mitotic spindle formation, chromosomal stickiness, impaired chromosomal separation, chromosomal translocations, chromosomal break, inversion of chromosomal segments and chromosomal fragmentation.[24,30-33] Nonetheless, the root extract's phenolics and flavonoids contents may modulate these cytological abnormalities, and thus increase the antimitotic or antigenotoxic activity.[26, 31-37]

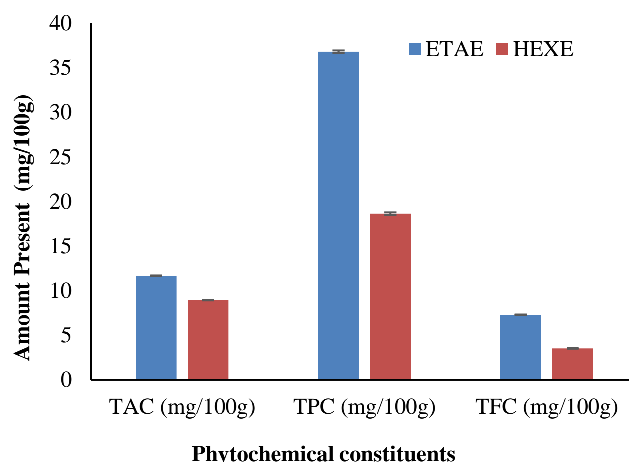


**Figure 1:** Phytochemical content in the root extracts of *Calotropis procera.*

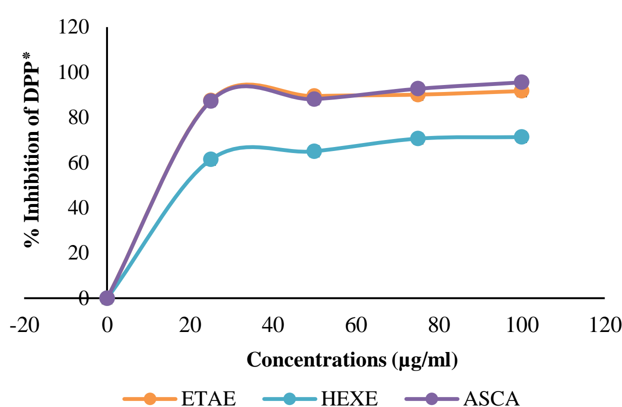


**Figure 2:** Free radical Scavenging activity of the root extracts of *C. procera* using DPPH assay.

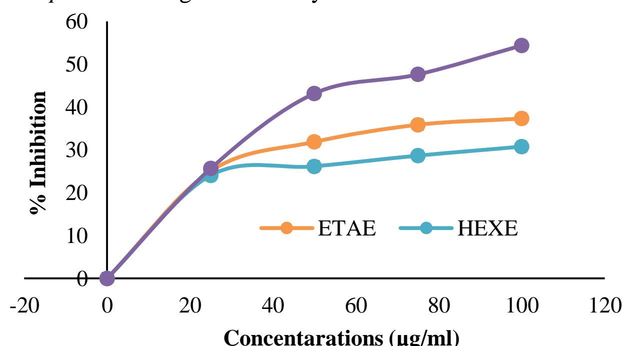


**Figure 3:** The redox activity of the root extracts of *C. procera* using FRAP assay.

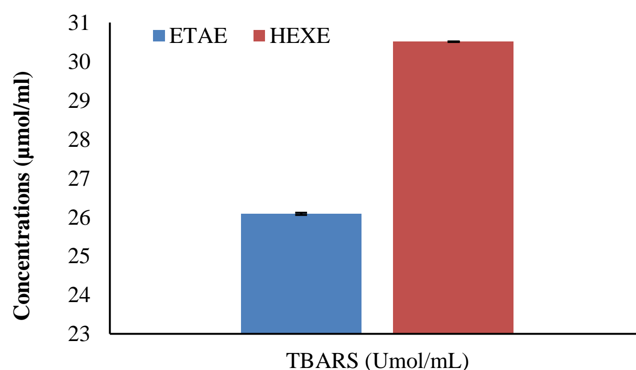


**Figure 4:** Antioxidant potential of the root extracts of *C. procera* using TBARS assay.


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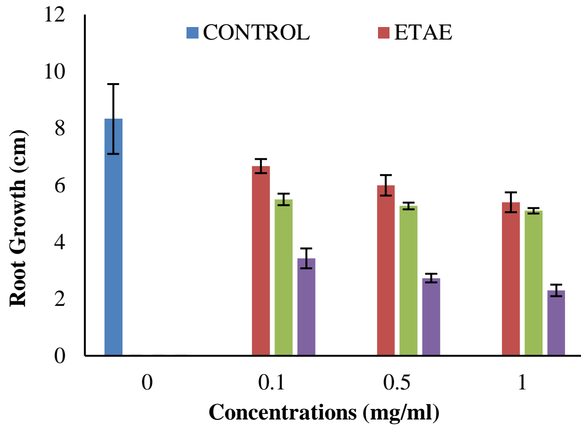


**Figure 5:** Formalin and root extracts effects on meristematic root growth inhibition.

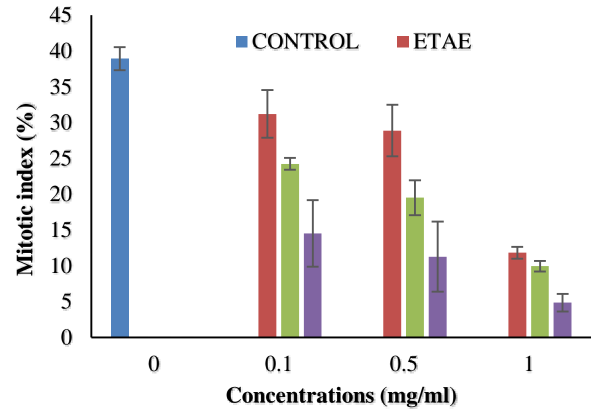


**Figure 6:** Formalin and root extracts of *C. procera* mitotic index outcome.

**Table 1:** Microscopic observations of the effects of the plant's root extracts and Formalin on *Allium cepa*

| | 24hrs | 48hrs | 72hrs |
|---|---|---|---|
| Control (Distilled water) | Normal cells with different stages of mitotic division | Normal cells with different stages of mitotic division | Normal cells with different stages of mitotic division |
| ETAE | | | |
| 0.1mg/ml | Distinct cells with different stages of dividing cells observed 5% were slightly enlarged. | Cells enlarged and elongated. Few dividing cells observed. | Enlarged and elongated, no dividing cells. |
| 0.5mg/ml | Dividing cells, Enlarged and elongated cells with 10% degeneration. | 25% cells degeneration. Few aberrations observed. | Degenerated cells. |
| 1.0mg/ml | Some dividing cells. Enlarged cells observed. | Cell degeneration observed. | Degenerated cells. |
| HEXE | | | |
| 0.1mg/ml | Dividing cells with distinct nucleus. Others were elongated and enlarged. | Distinct and few dividing cells. | No dividing cells. |
| 0.5mg/ml | Few dividing cells. Others were elongated and enlarged. | Enlarged & elongated cells. | Degenerated cells. |
| 1.0mg/ml | No distinct nor dividing cells. | No distinct nor dividing cells. | No distinct nor dividing cells. |
| Formalin | | | |
| 0.5mg/ml | Distinct and few dividing cells with few elongated. | Few dividing cells. Others were Elongated. | No dividing cells rather degenerated cells observed. |
| 1.0mg/ml | Few dividing cells. | Degenerating cells. | Degenerated cell. |

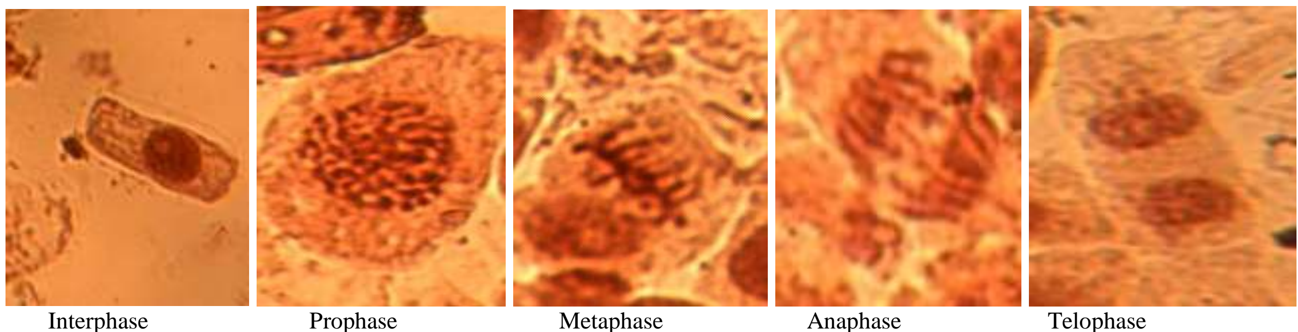


**Figure 7 (a):** Photomicrographs showing the normal mitotic division stages of the control group (Distilled water)


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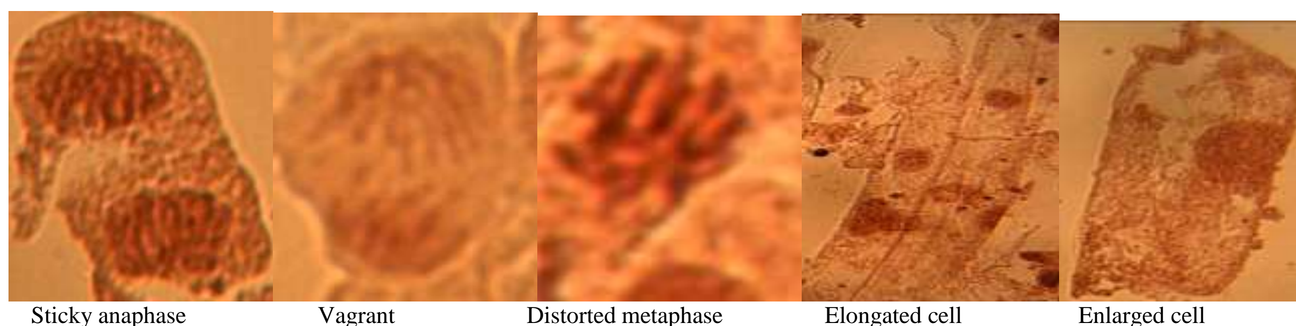


**Figure 7 (b):** Representative photomicrographs of the chromosomal aberrations in the ETAE group

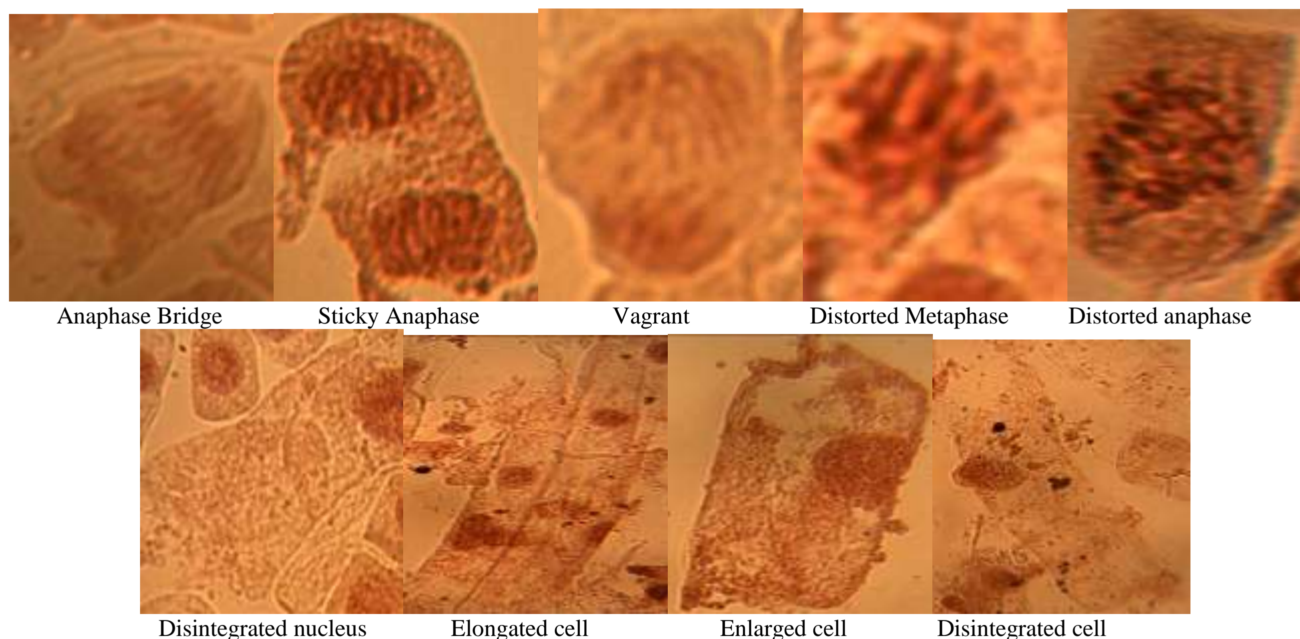


**Figure 7 (c):** Representative photomicrographs of the chromosomal aberrations in the HEXE group.

## Conclusion

This study investigated the cytotoxic and antioxidant activity of the root extracts of *C. procera,* to provide beneficial information to assist practitioners and users. The root extract's strong anti-free radical activity may be linked to the substantial concentration of phenolics content. The cytological abnormalities which may be caused by the interaction of the plant's alkaloid content with the onion's chromosomal apparatus, may be modulated due to the huge presence of total phenolics content and thus improves its anti-cytotoxic activity. Given the present toxicological outcome, further validation would be recommended in future study.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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## References

1. Ihegboro GO, Alhassan AJ, Ononamadu CJ, Sule MS. Identification of bioactive compounds in ethylacetate fraction of *Tapinanthus bangwensis* leaves that ameliorate $CCl_4$-induced hepatotoxicity in Wistar rats. Toxicol Res and Appl. 2020; 4:1–10.
2. Mumie-Bosch S and Pinto-Marijuan M. Free radicals, oxidative stress and Antioxidants. Encycl Appl Plant Sci. 2016; 1:16-19.
3. Hidayati MD and Rahmatulloh A. Antioxidant activity of *Uncaria gambir* (Hunter) Roxb Extracts. Trop J Nat Prod Res. 2022; 6(8):1215-1218.
4. Komanja IT, Mbaria JM, Gothumbi PK, Mbanbu M, Kobasa JD, Kiama SG. Cytotoxicity of selected medicinal plants extracts using the Brine shrimp lethality assay from Samburu country, Kenya. J Med Res. 2018; 4(5):249-255.
5. Ononamadu CJ, Alhassan AJ, Ibrahim A, Imam AA, Ihegboro GO, Owolarafe TA, Ezeigwe OC, Atiku MK, Sule MS. Toxicological study of aqueous methanol solvent fraction of methanol extract of *Dacryodes edulis* leaves. Toxicol Rep. 2020; 7(2020):909-918.
6. Owolarafe TA, Salawu K, Ihegboro GO, Ononamadu CJ, Alhassan AJ, Wudil AM. Investigation of cytotoxicity potentials of different extracts of *Ziziphus mauritiana* (Linn) leaf *Allium cepa* model. Toxicol Rep. 2020; 7(2020):816-821.
7. Ihegboro GO, Ononamadu CJ, Owolarafe TA, Iko Shekwolo. Screening for toxicological and anti-diabetic potential of n-hexane


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 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)


extract of *Tapinanthus bangwensis* leaves. Toxicol Res and Appl. 2020; 4:1-11.

8. Michael GS. Plant Morphology, 2nd Edition, Plant systematics, Academic press, Elsevier Inc, USA. 2010; 669-673.
9. Ihegboro GO, Ononamadu CJ, Owolarafe TA, Fadilu M, Okoro EJ. Anti-reno-haematological tenacity of *Calotropis procera* aqueous-methanol root extract in alloxan-induced pancrotoxic Wistar rats. Comp Clin Pathol. 2022; 3(1):1-9.
10. Ihegboro GO, Owolarafe TA, Ononamadu CJ, Bello H, Akpan MK. *Calotropis procera* Root Extract's Anti-diabetic and Hepatoprotective Therapeutic Activity in Alloxan-Induced Pancrotoxic Wistar Rats. Iranian J Toxicol. 2022; 16(4): 285-296.
11. Gaurav P and Neelam B. Calotropis procera: A phytochemical and pharmacological review. TJPS. 2018; 40(3):115-131.
12. Ihegboro GO, Alhassan AJ, Owolarafe TA, Ononamadu CJ, Salawu K, Afor E, Zaharaddeen IK, Edonyabo MD. Nutmeg toxicity: ameliorative effect of aqueous extract of *Guiera senegalensis* in experimental rat model. Ife J Sci. 2019; 21(2):001-014.
13. Niama S, Muhammad RK, Maria S. Antioxidant activity, Total phenolic and Total Flavonoid contents of whole plant extracts of *Torilis leptophylla* L BMC complement and Altern Med. 2012; 12: 221.
14. Nirmala P, Pramad KJ, Pankaj PR, Sangeeta R. Total phenolic, Flavonoid contents and Antioxidant activities of fruit, seeds and Bark extracts of *Zanthoxylum armatum* DC. Hindawi. 2020; 1-7.
15. Nantongo JS, Odoi JB, Abigaba G, Samson G. Variability of Phenolic and Alkaloid content in different plant parts of *Carissa edilis* vahl and *Zanthoxylum chalybeum* Engl. BMC Res Notes. 2018; 11:125.
16. Nithianan K, Shyamala M, Chen Y, Lathan LY, Jothy SL, Sasidharan S. Hepatoprotective potential of *Clitoria ternatea* leaf extract against paracetamol-induced damage in mice. 2011; 16(12):10134-10145.
17. Kristanti AN, Aminah NS, Zahroh FF, Hudaniah KB, Budiman MA, Renaldy R, Indrawan, Alfatsyah R, Wardana AP, Takaya Y. Phytochemistry of Syzygium polycephalum. Trop J Nat Prod Res. 2022; 6(5):728-731.
18. Ononamadu CJ, Alhassan AJ, Ibrahim A, Ihegboro GO, Imam AA, Owolarafe TA, Sule MS. *In vitro* and *in vivo* anti-diabetic and anti-oxidant activities of methanolic leaf extracts of *Ocimum canum.* Caspian J Intern Med. 2019; 10(2):162-175.
19. Ihegboro GO, Alhassan AJ, Ononamadu CJ, Owolarafe TA, Sule MS. Evaluation of the biosafety potentials of methanol extracts/fractions of *Tapinanthus bangwensis* and *Moringa oleifera* leaves using Allium cepa model. Toxicol Rep. 2020; 7(2020):671-679.
20. Shashank K, Ashutosh G, Abbay KP. Calotropis procera root extract has the capability to combat free radical mediated Damage. ISRN pharmacol. 2013; 1-8.
21. Sadeghi Z, Valizadeh J, Azizian SO, Akaberi M. Antioxidant activity and total phenolic content o fBoerhavia elegans (choisy) grown in Baluchisian. Iran Aricamia J Phytomed. 2015; 5(1):1-9.
22. Suman C, Shabana K, Bharathi A, Hemant L, Min HY, Mahmoud AE, Ikhlas AK. Assessment of total phenolic and flavonoid content, Antioxidant properties and yield of Aeropomically and conventionally Grown leafy vegetables and fruit crops: A comparative study. Evidence-based complement and Altern Med. 2014; 2014:1-9.
23. Adriana MFO, Lilian SP, Charlane KSP, Wemerson NM, Roosevelt AG, Otemberg SC, Maria FVS, ReinaldoNA, Temilce SA. Total phenolic content and antioxidant activity of some Malvaceae family Species. Antioxidants. 2012; 1:33-43.
24. Sanjiv K, Ragat S, Sadarshan O. Evaluation of antioxidant activity and total phenol in different varieties of *Lantana camara* leaves. BMC Res notes. 2014; 7:560.
25. Ihegboro GO, Owolarafe TA, Ononamadu CJ, Bello H, Akpan MK. *Calotropis procera* Root Extract's Anti-diabetic and Hepatoprotective Therapeutic Activity in Alloxan-Induced Pancreatic Toxicity in Wistar Rats. Iranian J Toxicol. 2022; 16(4):285-296.
26. Ihegboro GO, Ononamadu CJ, Owolarafe AT, Afor E, Zaharadeen IK. Antioxidants in Plant extracts may contribute to the modulation of their toxicity: An insight with *Allium cepa* model. NISEB J. 2018; 18(2):92-104.
27. Seo CS, Jung MS, Shin HK, Lee MY. *In Vitro* and *In Vivo* genotoxicity assessments and phytochemical analysis of the traditional herbal prescription Siryung-Tang. Molecules. 2020; 27:4006.
28. Trapp KC, Hister CAL, Laughinghouse iv HD, Boligon AD, Tedesco SB. Determination of phenolic compounds and evaluation of cytotoxicity in *Plectranthus barbatus* using the *Allium cepa* test. Caryologia. 2020; 73(2):145-153.
29. Mayara CS, Aline FM, Hataanderson LCS, Jennifer VG, Danilo GNP, Gabriela LP, Ellen BDR, Mayara JCL, Raphael SA, Leonardo OB, Helcio RB, Viviane ML. Laurus nebilis L: Assessment of the cytotoxic and genotoxic potential of aqueous extracts by macronucleus and *Allium cepa* assays. Brazillian J Pharmaceut Sci. 2020; 56: e18302.
30. Rajneet KS, Avinas N, Jatinder KK. *Allium cepa* root chromosomal aberration assay: An efficient test system for evaluating genotoxicity of Agricultural soil. Int. J Sci Res. 2014; 3(8):245-250.
31. Ifeoluwa TO and Adekunle AB. Genotoxic and anti-genotoxic effect of aqueous extracts of *Spondias mombin* L., *Nymphea lotus* L and *Luffa cylindrica* L on Allium cepa root tip cells. Caryologia. 2013; 66(4):360-367.
32. Herrero O, Perez MJM, Fernandez FP, Carrajd LL, Peropadre A, Hadan MJ. Toxicological evaluation of three contaminants of emerging concern by using the Allium cepa test. Mutation Res. 2012; 743(2012):20-24.
33. Farhana R, Davinder S, Shivani A, Prabhjot K, Hameetpal K, Pallvi M, Jahangeer Q, Adarsh P, Astha B, Balbir S, Harpreet W, Saroj A. Modulation of atrazine-induced chromosomal aberrations and cyclin-dependent kinase by aqueous extract of *Roylea cinerea* (D.Don) Bai-llon leaves in *Allium cepa*. Scientific report. 2022; 12:12570.
34. Nneka AK, Youasphiree N, Moganavelli S. Cytogenotoxic and biological evaluation of the aqueous extract of *Grewia lasiocarpa:* An *Allium cepa* assay. South Afr J Botany. 2019; 125:371-380.
35. Hoda AM, Hala M, Nesma M. Anti-mutagenic potential of Algal extracts on chromosomal aberrations in *Allium cepa*. Acta Biol Hungarica. 2017; 68(2):137-149.
36. Leandrim de Oliveria AL, Da Silva DB, Lopes NP, Debonsi HM. Chemical constituents from red algal *Bostrychia radicans* (Rhodomelaceae). New amides and phenolic compounds. Quim Nova. 2012; 35:2186-2188.
37. Kayalvizhi K, Subramanian NV, Boopathy S, Kathiresan K. Antioxidant properties of brown seaweeds *Turbinaria ornala* (Tumer) J Agardh, 1848 and *Padina terrastromatica* (Hauck). J Biotechnol Sci. 2014; 2:29-37.



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