**Original Research Article**

**Chemical Composition, Genotoxic and Cytotoxic Activities of Methanol Fruit Extract of African Spice *Tetrapleura tetraptera***

**ABSTRACT**

|  |
| --- |
| *Tetrapleura tetraptera* is a medicinal plant in most West Africa communities with a widespread medicinal use such as inflammation, convulsions, leprosy, asthma, hypertension, schistosomiasis, and malaria, antimicrobial, neuromuscular and anti-ulcerative effects. This study investigated the phytochemical composition as well as the genotoxic and cytotoxic effects of methanol extract from the fruit of *T. tetraptera* on the root cells of *Allium cepa* (onion). Onion bulbs were treated with extract concentrations of 2.5, 5.0, and 10 mg/mL, while tap water served as a negative control and Methotrexate (0.1 mg/mL) as a positive control. Results revealed a statistically significant (p < 0.05), concentration-dependent inhibition of root growth compared to the negative control. Cytological analysis indicated that all tested concentrations of the extract exerted cytotoxic effects, as evidenced by a reduction in mitotic index and the presence of chromosomal abnormalities, including micronuclei (MNC), chromatin bridges, binucleated cells, membrane damage, ghost cells, and cell death. Phytochemical screening identified the presence of flavonoids, alkaloids, saponins, tannins, and cardiac glycosides. Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed major constituents such as 4H-pyran-4-one, 3,3-dihydro-3,5-dihydroxy-6-methyl (26.85%), 5-hydroxymethylfurfural (18.13%), 3-O-methyl-D-glucose (16.52%), and 1,3-propanediol, 2-(hydroxymethyl)-2-nitro- (5.20%). These findings suggest that the bioactive constituents of *T. tetraptera* fruit extract possess significant cytotoxic and genotoxic potential in *A. cepa* root meristem cells. |

***Keywords****: Tetrapleura tetraptera* fruit extract; chemical composition; genotoxicity; cytotoxicity; *Allium cepa*; African spice; GC-MS.

**1. INTRODUCTION**

Phytochemicals are naturally occurring secondary metabolites produced by plants, many of which have been shown to possess significant nutritional, therapeutic, and pharmacological potential (Bhatti et al., 2022; Nwozo et al., 2023). These compounds contribute to the plant’s defense and ecological interactions and occur in diverse chemical classes namely: alkaloids, flavonoids, phenolics, tannins, saponins, and steroids (Koche et al., 2016; Awuchi, 2020).

In traditional medicine systems, they display a wide array of bioactivities such as antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic, and antimutagenic effects (Patra 2012; Roy et al., 2019; Wani et al., 2023). Such pharmacological properties support the historical use of medicinal plants and highlight the importance of investigating traditional remedies to identify novel therapeutic agents.

*Tetrapleura tetraptera* (Schum. & Thonn.) Taub. (Fabaceae) is a deciduous tree native to the West African region. The plant is widely used in African ethnomedicine and culinary traditions. Its fruit, which contains aromatic, oily seeds, is employed both as a spice and for its medicinal attributes (Adesina et al., 2016; Kemigisha et al., 2018; Bonsou et al., 2022). In various West African communities, different parts of the plant such as fruit, seeds, and flowers are utilized for their therapeutic properties in treating a range of ailments such as inflammation, convulsions, leprosy, asthma, hypertension, schistosomiasis, and malaria (Adesina et al., 2016; Sikam et al., 2022). Other reported ethnopharmacological uses include antimicrobial, hypoglycaemic, neuromuscular, molluscicidal, trypanocidal, and anti-ulcerative (Kuate et al., 2015). The plant is also used postpartum to promote uterine involution and is incorporated in the preparation of soups and herbal infusions for fever, gastrointestinal disorders, and constipation (Addo-Beatson, 2018).

The continued reliance on *T. tetraptera* in traditional healthcare across West Africa underscores the need for systematic ethnopharmacological evaluation. Its widespread use and reported bioactivities suggest the presence of potent phytochemicals that warrant detailed phytochemical and biological investigations. This study therefore, aims to examine the phytochemical composition, genotoxic and cytotoxic effects of the fruit methanol extract to understand its biological effects.



**Figure 1: Photo of *Tetrapleura tetraptera* fruit**

**2. material and methods**

**2.1 Plant collection**

*Tetrapleura tetraptera* fruits waspurchased from Afaha market in Uyo, Akwa Ibom State of Nigeria, in July 2023. The plant was identified and authenticated by the Department of Botany and Ecological Studies, University of Uyo, Nigeria.

**2.2** **Preparation of extract**

The sample was washed, dried in the shade, and ground using a laboratory mill. Extraction was conducted using a standard method (Umeh et al., 2005). Sixty grammes (60 g) of the powdered material was extracted in methanol for 48 hours. The solution was filtered and evaporated to dryness to obtain the methanol extract. The extracts were weighed, and the percentage yield was calculated.

**2.3** **Phytochemical Analysis**

Preliminary phytochemical screening for the detection of flavonoids, alkaloids, saponins, tannins and cardiac glycosides were conducted according to standard procedures (Ouandaogo et al., 2023). The presence or absence of a colour change indicated of the preliminary confirmation a class of phytochemicals (Enin et al., 2025)

**2.4 *Allium cepa* test.**

Small onions bulbs*, A. cepa*, were procured from Itam metropolitan market, Itu in Akwa Ibom State, Nigeria. The bulbs were 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 dissolve each of the extract (20 g) which were thereafter diluted to different concentrations (2.5, 5.0 and 10 mg/mL) respectively from the stock solution. Test concentrations of the fruit extract at 2.5, 5.0, and 10.0 mg/mL concentrations were prepared in 50 mL beakers and arranged in a series of 5 per test concentration. The experiment was therefore conducted following the method previously reported using tap water and Methotrexate (0.1 mg/mL) as negative and positive control respectively (Grant, 1994; Ikechukwu *et al.,* 2024).

**2.5 Microscopy**

To the root tips each in a test tube was added 1N HCl and the mixture was heated at 50ºC for 6 minutes to ensure maceration. They recovered, placed on a microscopic slide with a blank background and cut off at terminal tips. Orcein (2% w/v; 2 drops) was added and the mixture was stirred properly using a spatula. A cover slip was placed at 45º to avoid air bubbles and the cells were squashed by placing a filter paper on the cover slip and pressed lightly 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 depict chromosomal alteration. The mitotic index (MI) and frequency of chromosomal aberration (FCI) were calculated based on the number of aberrant cells per total cells counted at each concentration of the test extract (Bakare *et al.,* 2000; Magnus *et al.,* 2024). The mitotic inhibition was calculated using the following formula:

**2.6 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis**

GC-MS analysis of the extract was conducted using a Shimazu model of a Japan made GC-MS instrument (length of column = 30 m; diameter = 0.25 m and thickness = 0.25 m). Sample injection volume (1 µL) at a split ratio of 10:1 was utilized. Temperature of the oven started from 60 °C, increased to 180 °C before a ramp up to 250 ˚C. The GC-MS data analysis adopted the NIST template for its interpretation as previously reported by Kadhim et al. (2016).

**2.7 Statistical Analysis**

Data were statistically analyzed using one– way ANOVA then Tukey-Kramer multiple comparison test using Instat Graphpad software purchased from San Diego, USA. Differences between means were accepted as significant (i.e., p≤ 0.05).

**3. results and discussion**

**3.1****Plant Extraction**

The methanol extraction of 60 g of *T. tetraptera* fruit afforded 16.2 g, indicating a 26.8 percentage yield.

**3.2****Qualitative Phytochemical Analysis**

The results of the qualitative phytochemical analysis of *T. tetraptera* methanol extract revealed the presence of alkaloid, flavonoid, tannins, saponins and cardiac glycosides.

**3.3 Physicochemical Characterization**

The cytotoxic activity of T. tetraptera fruit extract on growing root number and length are presented (Table 1). A significant effect in the growth of roots compared to the control group was observed. The effect was dose-dependent because we observed that the highest concentration of the fruit extract exhibited total inhibition of the root growth. Methotrexate was the positive control and it afforded an average root length of 0.10±0.01 cm whereas the average root length was 4.36±0.24 in the negative control. At fruit extract concentration of 5.0 mg/mL, the average root length was 2.12±0.06 cm, however, at 10.0 mg/mL, the as no root growth (average root growth = 0.00±0.00). Noticeable changes such as changes in the root morphology to pale yellow while root tips turned brown as observed at the treatment with fruit extract at concentration of 2.5 and 5.0 mg/mL (Table 1).

**Table 1: Cytotoxicity of *T. tetraptera* fruit extract on growing roots of Onion**

|  |  |  |  |
| --- | --- | --- | --- |
| Treatment group | Concentration of extract (mg/mL) | Average root Number ± S. D | Average root length (cm)± S. D |
| Negative control | Tap water | 26.40±3.82 | 4.36±0.24 |
| Methotrexate | 0.1 | 2.10±0.02a | 0.10±0.01a |
| Extract | 2.5 | 20.16±2.24a | 3.16±0.94a |
| 5.0 | 10.30±1.02a | 2.12±0.06a |
| 10.0 | 0.00±0.00a | 0.00±0.00a |

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

**3.4 Cytogenetic Analysis**

The fruit extract caused a dose-dependent effect with a significant (p < 0.05) decrease in the mitotic index compared to the negative control. At concentration of 10 mg/mL, *T. tetraptera* fruit extracts exhibited mitotic index of 0.00±0.00 while the negative control group exhibited a mitotic index of 70.80±3.22 (Table 2).

**Table 2: Dividing and total cells under microscopic conditions**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Treatment group | Concentration of extract (mg/mL) | Total Number of cells | Dividing cells | M.I (%)± S. E |
| Negative control | Tap water | 500 | 354 | 70.80±3.22 |
| Methotrexate | 0.1 | 500 | 14 | 2.80±0.10a |
| Extract | 2.5 | 500 | 66 | 13.20±0.87a |
| 5.0 | 500 | 47 | 9.40±1.22a |
| 10.0 | - | - | - |

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

Table 3 present the genotoxic changes caused by the extract. Genetic and cellular changes were observed in all the groups albeit, with varied levels of changes. Scanning of the chromosome revealed that there were bridges and nuclear alteration particularly at higher extract doses (Table 3; Figure 2A). These changes were statistically relevant (p < 0.05) as we relate to the negative control. It was observed that there was no mutagenic fragments or breaks detected at any concentration of the fruit extract (Table 3). Gummy metaphase was identified (Figure 2B) but grow prominent with the group treated with the least dose of the fruit extract (2.5 mg/mL). Overall, the occurrence of structural and nuclear changes was observed to increase with increase extract dose (Table 3). The methotrexate-treated group however, experience the highest distortion. Further evidence of the fruit extract genotoxic potential was revealed by the induction of micronuclei in *Allum cepa* root meristem cells. Micronucleus formation did not show a dose-dependent pattern. However, both the methotrexate group and the extract-treated group (2.5 mg/mL) exhibited significant higher numbers of micro-nucleated cells compared to the negative control (p < 0.05) (Figure 2D). Moreover, cells with damaged membranes (Figure 2A and 2C), binucleated cells (Figure 2C and 2D), and nuclei showing damage (Figure 2C, 2E, and 2F) were at varying frequencies. Apoptotic cells were also revealed in the extract-treated groups (Figure 2D, 2E, and 2F).

**Table 3: Chromosomal and mitotic changes in the root cells of *Allium cepa***

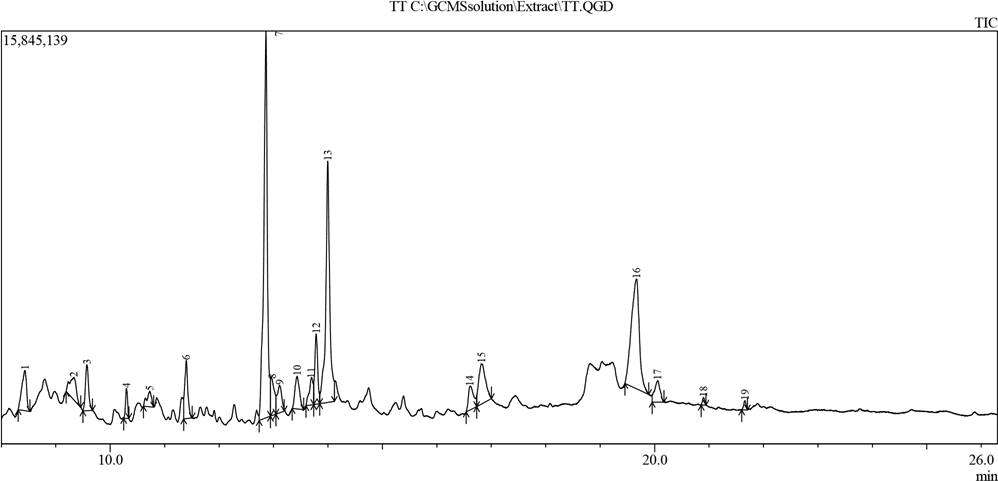
|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Treatment group | Concentration of Extract (mg/mL) | Chromosome breaks (%±S.E) | Stickiness (%)±S.E | Polar deviation (%)±S.E | Aberrant cells (%)±S.E | MNC (%)±S.E |
| Negative control | Tap water | - | 0.28±0.02 | 0.15±0.01 | 2.03±0.13 | - |
| Methotrexate | 0.10 | 3.23±1.38a | 34.12±2.38a | 15.23±2.34a | 51.12±3.42a | 3.12±0.56a |
| Extract | 2.5 | - | 4.19±0.33a | - | 22.31±2.18a | 2.01±0.22a |
| 5.0 | - | 8.28±0.34a | - | 35.42±3.16a | 0.89±0.01a |
| 10.0 | - | 10.17±2.34a | - | 52.38±4.28a | 0.02±0.01a |

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

**Figure 2: Photomicrograph showing the mitotic and chromosomal aberrations of *Allium cepa* root meristem cells after *T. tetraptera* fruit extract treatments under light microscope X40 magnification. Arrows indicate (A) Bridge, sticky chromosome and nuclear damage (B) sticky metaphase (C) bridge, laggard, cell wall damage, binucleated cells (D) Nuclear and membrane damage, apoptotic cells, binucleated cells (E) Nuclear and membrane damage, dead cells and apoptotic bodies (F) dead cells, membrane and nuclear damage**

**3.5 GC-MS Analysis**

The Gas Chromatography-Mass Spectrometry analysis of methanol extract of *Tetrapleura tetraptera* revealed nineteen compounds its chromatograph (Figure 3). Values of their retention time (minutes), concentration (%), and molecular weight (g/mol) are shown (Table 4). These compounds were: 2-Furanmethanol, dl-Glyceraldehyde dimer, cyclohexanone, 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, 2-Hydroxy-gamma-butyrolactone, 2,5-Dimethyl-4-hydroxy-3(2H)-furanone, 4H-pyran-4-one, 3,3-dihydro-3,5-dihydroxy-6-methyl, glyceraldehyde, 2(3H)-Furanone, dihydro-4-hydroxy, 1,6-Diazabicyclo(3.1.0)haxane-5-carboxylic acid-, methyl ester, 1,2-Ethanediol, 1-(2-furanyl)-, N-Aminopyrrolidine, 5-Hydroxymethylfurfural, tetrahydro-4H-pyran-4-ol, 1,3-propanediol, 2-(hydroxymethyl)-2-nitro-, 3-O-Methyl-d-glucose, di-n-octyl phthalate, 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester, 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione

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**Figure 3:GC-MS Chromatogram of *Tetrapleura tetraptera* Fruit Extract**

**Table 4: GC-MS Analysis of Methanol Fruit Extract of *Tetrapleura tetraptera***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Peaks** | **RT** | **Name** | **MF** | **MW** | **Area %** |
| 1 | 8.434 | 2-Furanmethanol | C5H602 | 98 | 3.57 |
| 2 | 9.330 | dl-Glyceraldehyde dimer | C6H12O6 | 180 | 2.73 |
| 3 | 9.573 | Cyclohexanone | C6H10O | 98 | 2.74 |
| 4 | 10.300 | 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one | C6H8O4 | 144 | 1.13 |
| 5 | 10.726 | 2-Hydroxy-γ-butyrolactone | C4H6O3 | 102 | 1.35 |
| 6 | 11.398 | 2,5-Dimethyl-4-hydroxy-3(2H)-furanone | C6H8O3 | 128 | 3.36 |
| 7 | 12.860 | 4H-pyran-4-one, 3,3-dihydro-3,5-dihydroxy-6-methyl | C6H8O4 | 144 | 26.28 |
| 8 | 12.975 | Glyceraldehyde | C3H6O3 | 90 | 2.59 |
| 9 | 13.113 | 2(3H)-Furanone, dihydro-4-hydroxy | C4H6O3 | 102 | 2.37 |
| 10 | 13.435 | 1,6-Diazabicyclo(3.1.0)haxane-5-carboxylic acid-, methyl ester | C6H10N2O2 | 142 | 2.79 |
| 11 | 13.690 | 1,2-Ethanediol, 1-(2-furanyl)- | C6H8O3 | 128 | 2.11 |
| 12. | 13.784 | N-Aminopyrrolidine | C4H10N2 | 86 | 3.79 |
| 13 | 13.996 | 5-Hydroxymethylfurfural | C6H6O3 | 126 | 18.13 |
| 14 | 16.605 | Tetrahydro-4H-pyran-4-ol | C5H10O2 | 102 | 2.38 |
| 15 | 16.819 | 1,3-propanediol, 2-(hydroxymethyl)-2-nitro- | C4H9NO4 | 151 | 5.20 |
| 16 | 19.664 | 3-O-Methyl-d-glucose | C7H14O6 | 194 | 16.52 |
| 17 | 20.054 | Di-n-octyl phthalate | C24H38O4 | 390 | 2.21 |
| 18 | 20.897 | 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester | C16H22O4 | 278 | 0.31 |
| 19 | 21.661 | 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione | C17H24O3 | 276 | 0.43 |

RT = retention time; MF = molecular formular; MW = molecular weight.

**4. DISCUSSION**

The study evaluated the genotoxic and cytotoxic effects of methanol fruit extract of T. tetraptera on root growth and root morphology. Various concentrations of the fruit extract exhibited inhibition of root growth with statistical relevance compared to control group (Table 1). Changes in colour were in observed in the root upon application of the extract. For example, roots showed pale yellow, pale brown and brown colours. Mutagenic break, stickiness, shrinkage and fragmentation were adopted to assess the cytotoxic and genotoxic effects. Methotrexate was the positive control and there was a significant decrease in the mitotic index (MI) of the *Allum cepa* cells treated with methotrexate (2.8%) as well as those treated with fruit extract of *T. tetraptera* compared to negative control (Table 2). Root growth inhibition directly depended on mitotic index such that increase or decrease in root growth showed a corresponding effect on the mitotic index (MI). Sumitha & Thoppil (2016) reported that lower mitotic index (<22%) than the negative control has fatal effect on the organism. A sublethal impact was observed below a 50 percentile (Šiviková & Dianovský, 2000). Mitotic index (MI) assesses the proportion of cells undergoing mitosis at M-phase and its blockage leads to cell death or a delay in cell proliferation rate (Gallway, 2000; Ligasová *et al.*, 2023).

A reduction in mitotic activity can hinder deoxyribonucleic acid (DNA) synthesis or interrupt the second gap phase (G₂) of the cell cycle, ultimately preventing cells from attaining mitosis (Swift & Golsteyn, 2014). The reduced mitotic index observed in *Allium cepa* roots exposed to *T. tetraptera* fruit extract could likely results from disruptions in the cell cycle or chromatin damage caused by interactions between the extract and cellular deoxyribonucleic acid. These findings suggest that the extract possesses both inhibitory and mitosis-suppressing effects, that affect root growth and cell division in *A. cepa*. This impact may be attributed to the extract’s cytotoxic bioactive components, which appear to suppress deoxyribonucleic acid synthesis and decrease the population of dividing root cells. Kashtwari et al. (2017) reported that chromosome agglomeration can result from the effect of toxic substances embedded in plant extracts. G’’om’’urgen (2005) explained that gumminess results from the effect of bioactive constituents on the physiochemical parameters of deoxyribonucleic acid, protein or both, on the formation of complexes with phosphates in deoxyribonucleic acid, induced DNA condensation, or create cross-links between or within chromatids.

In this study, chromosomal fragmentation was observed after treatment of *Allum cepa* with the fruit extract of *T. tetraptera* at varied concentrations. The extract interacts with the cell cycle, distorted chromatid organization or deoxyribonucleic acid synthesis and caused mutagenic breaks. Chromosome alterations increased significantly after exposure of the sample to the extract, indicating genotoxic activity (Table 3). The fruit extract of *T. tetraptera* did not induce the formation of multinucleated cells between 2.5 to 10 mg/mL. Chromosomal fragmentation may result from chromosome breaks, likely as a result of anaphase/telophase bridges (Sharma and Sen, 2002; Hawkins & Miles, 2021). Membrane damaged cells could be as a result of interactions with certain cytotoxic materials over a time. Multinucleated (MNC) and binucleated (BNC) cells were observed in extract-treated groups, possibly due to the prevention of cytokinesis or cell plate formation (Çelik & Aslantürk, 2009). Besides, some ghost cells were noted in certain frequencies in 2.5 -5.0 mg/mL treated groups. The activities of bioactive compounds of the extract could lead to nucleus damage and disruption of cytoplasmic structures resulting in ghost cells. In addition, theextract 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 doses of toxin, heavy metals chemical pollutants.

Several studies have reported that some classes of flavonoids, saponins, tannins and glycosides exhibit mutagenic and genotoxic effects. Manly, organic compounds of nitroaromatics, aromatic amines, aromatic ketones, polycyclic aromatic hydrocarbons and hydroxy compounds have been reported for these activities (Kelly et al., 2001; Rashwan et al., 2021; Beltzig et al., 2024). Results of our qualitative phytochemical analysis revealed that *T. tetraptera* fruit extract contained flavonoids, alkaloid, saponins, cardiac glycosides and tannins. Moreover, spectroscopic data from GC-MS analysis showed the presence of heteroatomic compounds of different nature such as alcohol and ketones (e.g., 2-Furamethanol and glyceraldehyde), ketones (e.g., 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one and cyclohexanone), cyclic and aromatic ketones, bicyclic amine compounds (e.g., 4H-pyran-4-one, 3,3-dihydro-3,5-dihydroxy-6-methyl and N-Aminopyrrolidine), aldehyde (e.g., 5-Hydroxymethylfurfural), ethers (e.g., tetrahydro-4H-pyran-4-ol) and esters (e.g., 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester and Di-n-octyl phthalate). We suggest that the presence of these compounds in the extract may be responsible for the observed cytotoxic and genotoxic activities in this study.

**4. Conclusion**

In this study, the fruit extract of *T. tetraptera* 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. As such, caution is advised when using this plant in traditional medicine, and high doses should be avoided due to possible harmful effects. The observed toxicity is likely linked to the phytochemical constituents present in the extract. However, we recommend that further research involving bioassay-guided fractionation and isolation of key compounds to identify the specific agents responsible for these effects.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declare that NO generative 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.

**COMPETING INTERST**

Authors have declared that no competing interests exist.

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