

Original Research Article

Genotoxic Potential, Total Flavonoid and Phenolic Contents of *Solanum anomalum* Thonn. ex Schumach Leaf

ABSTRACT

Solanum anomalum, a medicinal plant, used in ethnomedicine for their antimalarial, antidiabetic, and antiphlogistic activities was investigated for cytotoxic and genotoxic effects on the root meristem cells of *Allium cepa*. The extract and fractions was also screened for total flavonoid and phenol content using standard methods. Onion bulbs were exposed to 2.5 mg/mL, 5mg/mL, and 10 mg/mL concentrations of the leaf extract for macroscopic and microscopic analysis. Tap water was used as a negative control and Methotrexate (0.1 mg/mL) 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 have 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 extract treatment further induced cell death, ghost cells, cells membrane damage, and binucleated cells. The dichloromethane fraction was found to contain the highest level of total flavonoid and phenol followed by the crude extract. These results suggest that *Solanum anomalum* leaf extract possess cytotoxic and genotoxic effects on *A. cepa* which is as a results of its phytochemical constituents.

Keywords: *Solanum anomalum*; genotoxicity; cytotoxicity; *Allium cepa*

INTRODUCTION

Solanum anomalum Thonn. ex Schumach, Solanaceae, a plant whose fruits and leaves are used medicinally and nutritionally is commonly found growing in West and East Africa sub-regions. Its parts are utilised locally to treat diabetes, gastrointestinal disorders, infections, inflammation and pains (Burkill, 2000; Bukenya and Hall, 1988; Offor and Ubengama, 2015). Hypoglycemic and antidiabetic activities of the leaves have been reported (Okokon et al., 2022). More so, *in vivo* and *in vitro* antiplasmodial (Okokon et al., 2016; Okokon et al., 2017a), anti-oedema (Okokon et al., 2017b), antioxidant and antiulcer (Okokon et al., 2019a), anticonvulsant and depressant (Okokon et al., 2019b), analgesic (Okokon et al., 2020) and antidiarrhoeal (Udobang et al., 2022) properties of the leaf extract have also been

reported. Phytochemical constituents such as alkaloids, flavonoids, saponins, tannins, diosgenin, a diosgenin glycoside (25(R)-diosgenin-3-O- α -L-rhamnopyranosyl-(1 \rightarrow 4)- β -D-glucopyranoside, uracil, 5-methyluracil, 1-octacosanol, and octacosane have been reported on the leaves of the plant (Okokon *et al.*, 2016; Okokon *et al.*, 2022). Therefore this is aimed at investigating the genotoxic potentials, total flavonoid and total phenolic contents of the leaf extract.

Materials and Methods

Plants collection

Fresh leaves of *Solanum anomalum* were collected in compounds in Uruan area, Akwa Ibom State, Nigeria in August, 2022. The plant was identified and authenticated by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. Herbarium specimen was deposited at Department of Pharmacognosy and Natural Medicine Herbarium, University of Uyo (UUH.75a).

Extraction

Fresh leaves of *S. anomalum* were washed, cut into smaller pieces and dried under shade for two weeks. The leaves were further pulverized to powder using electric grinder. The powdered leaves were divided into two parts; one part (1.5kg) was macerated in 50% ethanol for 72 hours. While the other part, (1.5 Kg) was successively and gradiently macerated for 72 hrs in each of these solvents, n-hexane, dichloromethane, ethyl-acetate and n-butanol to give corresponding fractions of these solvents. The liquid filtrates obtained were concentrated and evaporated to dryness *in vacuo* at 40°C using rotary evaporator. The dried crude extract and fractions were stored in a refrigerator at 4°C until use for the proposed experiment.

***Allium cepa* test**

Small bulbs of the common onion, *A. cepa*, were procured from Jos, Plateau 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. The plant extract (20 g) was dissolved in 200 mL of distilled water. Different concentrations of 2.5 mg/mL, 5 mg/mL and 10 mg/mL respectively were prepared from the stock solution. Test concentration of the 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 (0.1mg/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.

The root tips were each placed in a test tube with 1N HCL and heated at 60 °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.

an Olympus CX21 binocular microscope. Photomicrographs were taken from good preparation using the Olympus CX21 binocular microscope fitted with an MD500 amscope microscope eyepiece camera.

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 as suggested by Grant (1982) and each slide was examined using a Light Olympus CX21 Microscope fitted with an MD500 Amscope microscope eyepiece camera at a magnification of ×40. Microphotographs were taken to show chromosomal aberrations. The mitotic index and frequency of chromosomal aberration were calculated based on the number of aberrant cells per total cells scored at each concentration of each sample (Bakare *et al.*, 2000).The mitotic inhibition was determined using the following formula:

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

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

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

ANALYSIS

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.

Determination of Total Phenolic Content

Total phenolic contents of the crude extract and fractions of the seed were determined spectrophotometrically with Folin-ciocalteu reagent. 0.5 ml (1 mg/ mL) of crude extract and fractions were mixed with 2.5 ml of 10% Folin-ciocaltau reagent and 2 ml of Na₂CO₃ (7%). The resulting mixture was then vortexed for 15 seconds incubated at 40°C for 30 minutes or colour development. The absorbance of the samples was measured at 765 nm wavelength. Follin-ciocalteu reagent (2.5 mL) was also added to different concentrations (20 – 100 µg/mL) for the calibration curve of gallic acid. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of gallic acid equivalent per gram dry weight. These performed in triplicates (Kaur and Kpoor, 2002; Madhu *et al.*, 2016).

DETERMINATION OF TOTAL FLAVONOID CONTENT

The total flavonoids content was estimated using the procedure described by (Madhu *et al.*, 2016). A total of 1mL of plant extracts and fractions were diluted with 200 µL of distilled water separately followed by the addition of 150 µL of sodium nitrate (5% solution). This mixture was incubated for 5 minutes and then 150 µL of aluminium chloride (10% solution) was added and allowed to stand for 6 minutes. Then 2 mL of sodium hydroxide (4% solution) was added and made up to 5ml with diluted water. The mixture was shaken well and left to stand for 15 mins at room temperature. The absorbance was measured at 510 nm. Appearance of pink colour showed the presence of flavonoid content. The total flavonoid content was expressed as rutin equivalent mg RE/g extract and fractions on a dry weight basis using the standard curve.

STATISTICAL ANALYSIS.

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

RESULTS

PHYSICOCHEMICAL CHARACTERIZATION.

The effects of *Solanum anomalum* 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 *Solanum anomalum* extract caused significant inhibition in the growth of roots in comparison to negative control and positive control. The inhibition of root number and root length was greater with increasing concentrations of the extract. The root length averages in negative and positive control (methotrexate) groups were 4.16 ± 1.26 and 0.25 ± 0.22 cm respectively. However, average root lengths in 10 mg/mL treatment group was decreased significantly compared to that of the negative control; 0.39 ± 0.19 and 0.44 ± 0.10 cm) for *S. anomalum* (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 nearly normal during the negative control treatment, but changes in appearance of the roots were observed in the groups treated with 2.5 – 10 mg/mL of *S. anomalum* leaf extract. The roots appearances turned from slight yellow at 5 mg/ml to slight brown at 10 mg/ml of the extract (Table 1).

CYTOGENETIC ANALYSIS

Table 2 shows the effects of *Solanum anomalum* leaf extracts on cytogenetic parameters of *Alium cepa* roots. Cytogenetic analysis performed showed that the extract caused concentration-dependent and significant ($p < 0.05$) decreases in the mitotic index when compared to that of negative control. The extract of *S. anomalum* at 10 mg/mL had mitotic index of 29.0 ± 8.34 as compared to 57.60 ± 12.34 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, and *Solanum anomalum* leaf extract-treated groups as depicted in Table 3. An analysis of chromosome aberrations observed showed that most of the fragments detected in the different treatments were of chromosome type especially in the highest concentration. The observation of chromosome breaks showed the clastogenic effect of extract. This was significant ($p < 0.05$) when compared to negative control group. Sticky metaphase and polar deviations (wrong directions of chromosome movement) were also observed (Figures 1(b) and 1(c)) in the extract-treated groups but were more frequent in the group treated with the highest concentration of the extract (10 mg/mL). Sticky metaphase features were also observed in the extract- treated groups. It was generally observed that these abnormalities increased with increasing concentrations of the extracts. 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 with the extract with the highest concentrations exerting the highest effects and higher frequencies of aberrations). However, the highest value of aberrant cells was observed in methotrexate-treated group (positive control)(Table 3). Genotoxic activities of the extracts were further demonstrated by the induction of micronuclei in the root tip meristem cells of *Allium 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. anomalum* had high numbers of cells with micronuclei in the test compared to negative control, which were statistically significant ($p < .05$). The increase occurred in the positive control. In *Allium cepa* test, a strong toxic effect of *S. anomalum* leaf extract was observed, supported by great occurrence of sticky metaphases, leading to cellular death (mitotic index decrease. In addition, cells with membrane damage (Figure 1(a)), binucleated cells (Figure 1(b)), and nucleus damage (Figures 1(c) and 1(d)) were found in various frequencies. Also, apoptotic cells (Figure 1(c)) were detected in the group treated with the extract.

Table 1: Cytotoxicity of *Solanum anomalum* 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	34.10 \pm 4.62	4.66 \pm 1.26
Methotrexate	0.1	8.16 \pm 3.28 ^a	0.25 \pm 0.22 ^a
<i>Solanum anomalum</i>	2.5	10.80 \pm 3.96 ^a	1.44 \pm 0.52 ^a
	5.0	22.20 \pm 12.59	0.73 \pm 0.25 ^a
	10.0	17.0 \pm 7.10 ^a	0.44 \pm 0.10 ^a

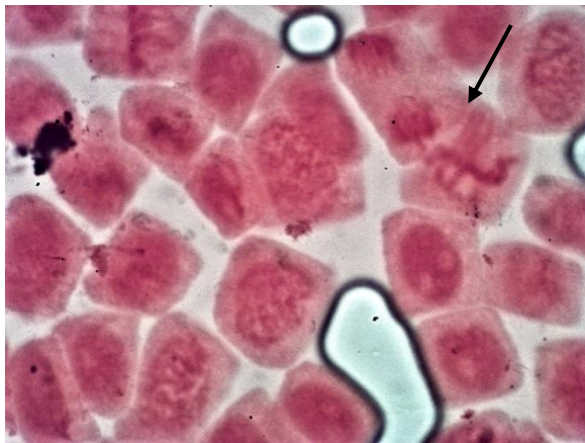
Values are expressed as mean \pm SEM (n=5). Significant at $p < 0.05$ when compared to negative control.

Table 2: Dividing and total cells counted under microscopic observations and mitotic values in control and treatment concentrations

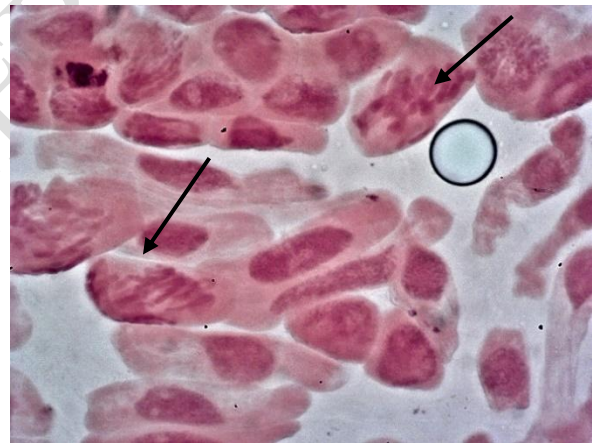
Treatment group	Concentration of extract (mg/mL)	Total Number of cells	Dividing cells	M.I (%) \pm S.E
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Negative control	Tap water	500	288	57.60±12.34
Methotrexate	0.1	500	15	3.00±0.68 ^a
<i>Solanum anomalum</i>	2.5	500	273	54.60±6.27
	5.0	500	187	37.40±6.79 ^a
	10.0	500	145	29.0±8.34 ^a

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



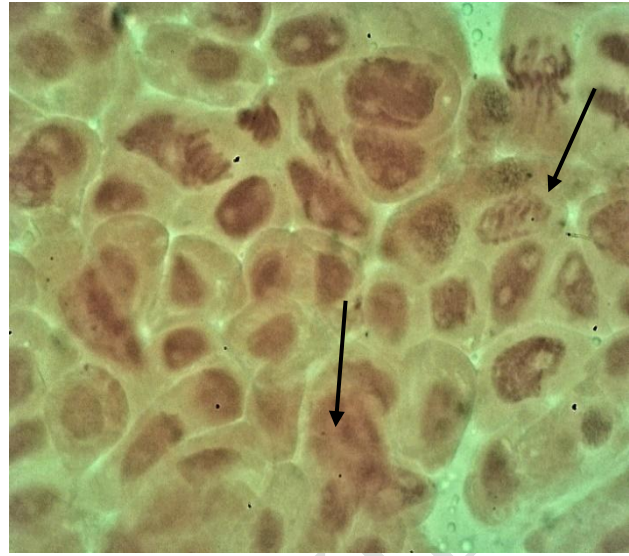
(a)



(b)



(c)



(d)

Figure 1: Photomicrography showing the mitotic and chromosomal aberrations after the *Solanum anomalum* extract treatments in *Allium cepa* root tip meristem cells visualized with light microscopy at magnification X40. (a) arrow indicates polar deviations; (b) arrow indicates chromatids bridges and stickiness; (c) arrow indicates stickiness; (b) chromosomal breaks and damaged nucleus.

UNDER PEER REVIEW

Table-3: Chromosomal and mitotic aberrations in the root meristematic cells of *Allium cepa* after treatment of extract of *Solanum anomalum*.

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	-	0.11±0.08	0.31±0.04	1.05±0.56	-
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>Solanum anomalum</i>	2.5	3.12±1.29 ^a	3.03±0.25 ^a	2.02±0.12 ^a	19.14±2.39 ^a	9.16±2.16 ^a
	5.0	4.33±1.36 ^a	14.25±3.28 ^a	6.36±1.28 ^a	28.48±6.28 ^a	1.02±0.38 ^a
	10.0	3.11±1.68 ^a	4.02±1.86	1.18±0.34 ^a	35.01±8.45	2.16±1.16 ^a

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

Total Flavonoids Content of *S. anomalum* extract and fractions

The result of total flavonoid content of leaf extracts and fractions of *Solanum anomalum* leaves presented in Figure 2 shows that the dichloromethane fraction contain the highest quantity of flavonoid followed by crude extract and ethyl acetate fraction.

Total Phenolic Content of *S. anomalum* extract and fractions.

The total phenolic content of the crude extract and fractions as deduced from the calibration graph showed that the dichloromethane fraction has the highest phenolic content (3.41 mg/g) followed by methanol fraction (0.76 mg/g) (Figure 3).

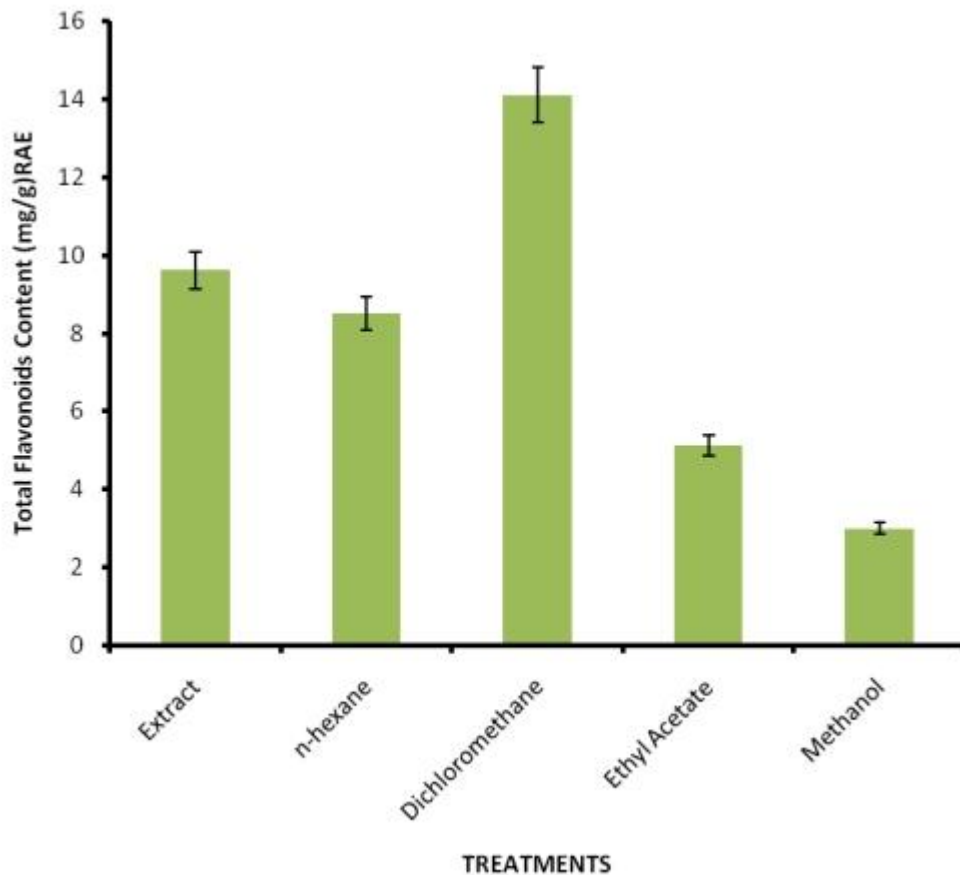


Figure 2: Total Flavonoids Content of *S. anomalum* extract and fractions

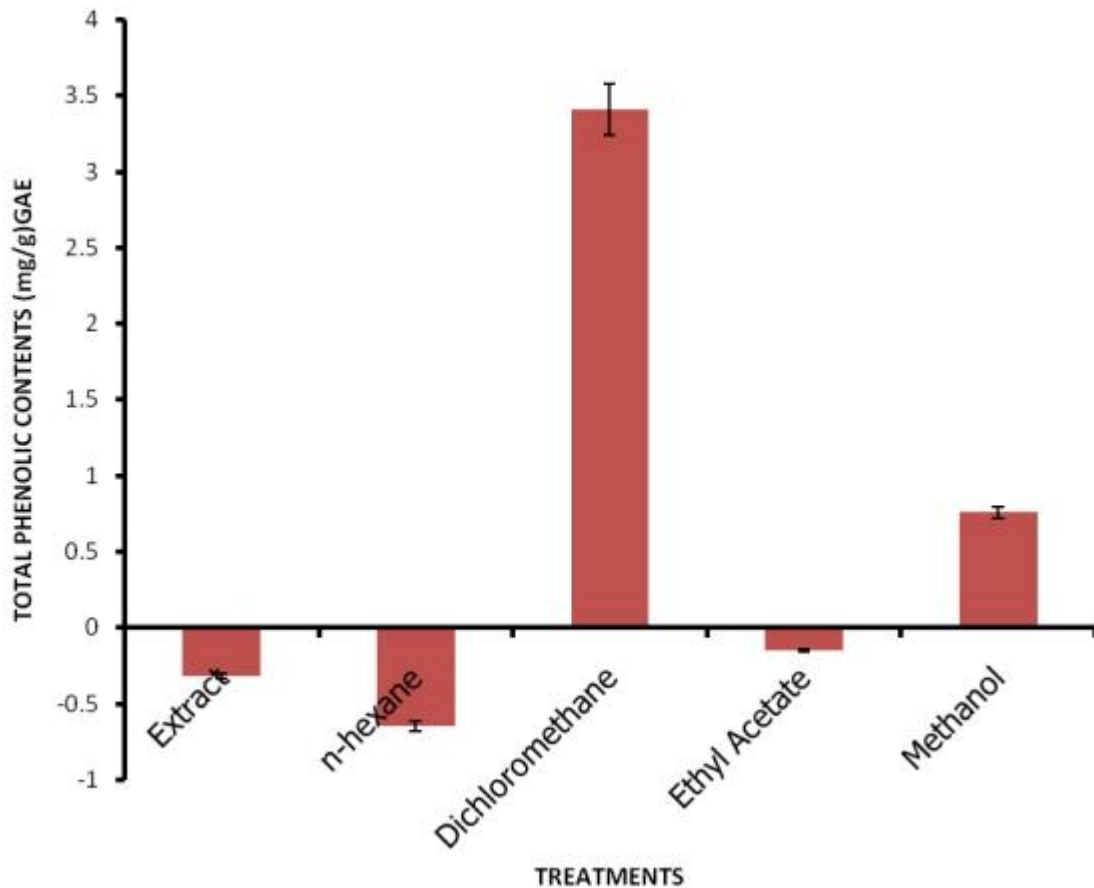


Figure 3: Total Phenolic Content of *S. anomalum* extract and fractions

Discussion

In this study, toxic effect of *Solanum anomalum* 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 (3.00 %) was significantly decreased when compared to control. Significant inhibition in the onion roots treated with *S. anomalum* leaf extract (54.60 %, 37.40 % and 29.0 % 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). The mitotic index (MI) which gives information about the total number of dividing cells in the cell cycle is used as a parameter to evaluate the cytotoxicity of an agent. A decrease or increase in MI determines the level of cytotoxicity of an agent (Fernandes *et al.*, 2007).

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.*, 2006; As *et al.*, 2007; Akinboro and Bakare, 2007; Chukwujekwu and Staden, 2014; Priyanka *et al.*, 2019). The decreased mitotic index in *A. cepa* roots treated with *S. anomalum* extracts 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 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 reinforces the hypothesis of the toxic effect of the extracts. 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; T’urgen, 2007). Chromosomal aberrations (CA) are changes in chromosome structure resulting from a break or exchange of chromosomal material. Chromosome aberrations provide important information and may be considered an efficient test to investigate the genotoxic potential of different agents and substances (Carita and Marin-Morales, 2008). 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. The extract used 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 indicates 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 were found to increase in the groups treated with 2.5 mg/mL of the 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 *Lavandula stoechas* and *Ecballium elaterium* (As *et al.*, 2007; As *et al.*, 2009), *Azadirachta indica* (Soliman, 2001) *Psychotria* species (Akinboro and Bakare, 2007).

In this study, membrane damage cells were observed in groups treated with 5 mg/mL and 10 mg/mL of the extract. These results show that the extract over certain concentrations may cause cytotoxicity as observed in 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 extract prevented 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 (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. Also, high concentrations (5 mg/ml and 10 mg/ml) of the extract 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.

The results of this study show that the extract of *S. anomalum* 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), 1(b), 1(c), and 1(d)), suggesting cytotoxic and genotoxic activities of the extract.

The results of this study showed that the extract contains a high phenolic and flavonoid content especially the dichloromethane fraction. Flavonoids such as quercetin have been reported to demonstrate mutagenic and genotoxic potentials in various studies (Ping *et al.*, 2017). The high phenols and flavonoids contents in the leaf extract must have contributed to the observed cytotoxic and genotoxic activities in this study.

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

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