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GC-MS Analysis, Genotoxic and *In vitro* Antioxidant Activities of Fruit Extract and Fractions of *Solanum anomalum* Thonn. Ex Schumach (Solanaceae)

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Abstract

Solanum anomalum Thonn. Ex Schumach, Solanaceae fruits are used in Ibibio traditional medicine for the treatment of various ailments including diabetes mellitus and malaria. The ethanol fruit extract and fractions of *S. anomalum* were investigated for cytotoxic and genotoxic effects on the root meristem cells of *Allium cepa* and *in vitro* antioxidant activity using various models; 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, Ferric Reducing Power assay (FRAP), Nitric oxide (NO) scavenging assay, total flavonoid and phenol content using standard methods. Onion bulbs were exposed to 2.5 mg/mL, 5 mg/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. Also, the GC-MS analysis of the fractions were carried out. 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 *Allium 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 fruit extract and fractions exhibited significant antioxidant activity with the hexane and dichloromethane fractions demonstrating higher antioxidant potentials. The dichloromethane fraction was found to contain the highest level of total flavonoid and phenol followed by the crude extract. The fruit fractions (hexane, dichloromethane and ethyl acetate) were found to contain some pharmacologically active compounds These results suggest that the fruit extract and fractions of *Solanum anomalum* possess genotoxic, cytotoxic and antioxidant potentials which are due to the activities of the phytochemical constituents.

Keywords: *Solanum anomalum*, antioxidant; Total phenol; Total flavonoid; GC-MS analysis.

1. Introduction

Solanum anomalum Thonn. ex Schumach (Solanaceae), is a herbaceous plant whose fruits and leaves are utilised for various purposes especially in traditional medicine and nutrition for the preparation of soups and sauces. It is distributed widely in West and East Africa sub-regions. Parts of the plant are utilised ethnomedically to treat diabetes, gastrointestinal disorders, malaria, infections, inflammation and pains [1]. Hypoglycemic and antidiabetic activities of the fruits and leaves have been reported [2, 3] The fruits extract which has been proven to offer significant protection against lead-induced liver, kidney and testicular injuries [4]; has oral LD_{50} confirmed to be above 5,000 mg/kg [2] and intraperitoneal LD_{50} of 2260 ± 131.78 mg/kg. Secondary metabolites such as saponins, cardiac glycosides, anthraquinones, terpenes, flavonoids, tannins and alkaloids have been reported in the fruits extract, while diosgenin, a diosgenin glycoside (25(R)-diosgenin-3-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside, uracil, 5-methyluracil, 1-octacosanol, and octacosane have been reported on the leaves of the plant [3] in which *in vivo* and *in vitro* antiplasmodial activities [5] have been reported. We report in this study the phytochemical analysis, genotoxic, cytotoxic and *in vitro* antioxidant activities of the fruits extract and fractions *S. anomalum*.

2. Materials and Methods

2.1. Plants Collection

Fresh fruits of *Solanum anomalum* were collected in compounds in Afaha Idoro village in Uyo area, Akwa Ibom State, Nigeria in August, 2023. The plant was identified and authenticated by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. Hebarium specimen was deposited at Department of Pharmacognosy and Natural Medicine Herbarium, University of Uyo (UUH.75a).

2.2. Extraction

Fresh fruits of *S. anomalum* were washed and dried under shade for two weeks. The fruits were further pulverized to powder using electric grinder. The powdered fruits material was divided into two parts; one part (1.5 kg) was macerated in 50% ethanol (7.5 L) for 72 hours at room temperature (28 ±2 °C). While the other part, (1.5 kg) was successively and gradiently macerated for 72 h in each of these solvents (2 x 5L), *n*-hexane, dichloromethane, ethyl-acetate and methanol to give corresponding fractions of these solvents. These were thereafter filtered and the liquid filtrates were concentrated and evaporated to dryness in *vacuo* 40°C using a rotary evaporator (BuchiLab, Switzerland). The extract and fractions were stored in a refrigerator at -4°C, until used for the proposed experiments.

2.3. Genotoxicity and Cytotoxicity Testing

2.4 Allium Cepa Test

Small bulbs of the common onion, *A. cepa*, were procured from Elele market in Elele, Rivers State of Nigeria. Prior to initiating the test, the bulbs were prepared with the outer scales of the bulbs and the dried bottom plates removed without injuring the root primordia using a small sharp knife. These were collected into a jar of water and discarded. The plant extract (20 g) was dissolved in 200 mL of distilled water. The different concentrations of the extract; 2.5 mg/mL, 5 mg/mL and 10 mg/mL were prepared from this stock for the study. The test concentrations of the fruit extract (2.5 mg/mL, 5 mg/mL, and 10 mg/mL) were prepared in five 50 mL beakers per concentration in a series, filled up for each concentration. One *A. Cepa* bulb was placed on top of each beaker, with the root primordia submerged in the test solutions. Tap water was used as negative control and Methotrexate (0.1 mg/mL) was used as positive control. After 24 hours, the test concentrations solutions were replaced by freshly prepared test concentrations of the extract in all test concentrations, methotrexate and distilled water. This continued for 72 hours.

2.5. Macroscopic Analysis

After 72 hours of treatments, all the roots of each bulb per beaker were counted in all the tested concentrations and mean root number was calculated in each concentration. Similarly, the roots' lengths of five longest roots per bulb in each concentration were measured using a metre rule and the mean root length was calculated. These were also done for the methotrexate treated group and control.

2.6. Root Harvest and Slide Preparation

Several root tips were cut at a length of 10 mm from the bulbs, and respectively fixed in 3:1 (v/v) ethanol: glacial acetic acid and 1N HCL before putting them in specimen bottles and storing in a refrigerator until use [6].

2.7. Microscopy

Each root tip was fixed and macerated by being placed in a test tube with 1N HCL and heated at 50°C for 6 minutes. 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 45º to avoid air bubbles. After that, 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 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" [7]. The mitotic inhibition was determined using the following formula:

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

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

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

The following parameters were used for determination of cytotoxicity and genotoxicity: (i) the mitotic index (MI) was calculated as the ratio between the number of mitotic cells and the total number of cells scored and expressed as percentage and (i) chromatin aberrations (stickiness, bridges, breaks and polar deviation) were used as endpoints for determination of cytogenetic effects and micronuclei (MNC) were scored in interphase cells per 500 cells.

2.8. *In-vitro* Antioxidant Studies

2.9 Determination of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

DPPH free radical scavenging of the fruit extract and fractions of *S. anomalum* and ascorbic acid prepared in methanol at concentrations of 20, 40, 60, 80 and 100 µg/mL were evaluated according to the method of Shekhar and Anju [8]. 1 mL of DPPH was added to 3 mL of the solutions prepared with the fruit extract and fractions (n-hexane, dichloromethane, ethyl acetate and methanol) and ascorbic acid and stirred for 1 minute. Each mixture was kept in the dark for 30 minutes and the absorbance (A_s) was measured at 517 nm. The assays were carried out in triplicates and the results expressed as mean values ± standard deviations. Lower absorbance of the reaction mixture indicated higher free radical activity. A similar procedure was repeated with methanol as blank, which served as control. The percent DPPH scavenging effect was calculated using the following equations:

DPPH scavenging effect (%) or Percent inhibition= $[(A_0 – A_s) / A_0] \times 100$.

Where A_0 is the absorbance of control reaction and A_s is the absorbance in the presence of test or standard sample (ascorbic acid) [8].

2.10 Ferric Reducing Power assay (FRAP)

This was determined using a method earlier described by Oyaizu [9]. Various concentrations 20, 40, 60, 80, and 100 µg/mL of the fruit extract and fractions of *S. anomalum* and ascorbic acid (1 mL) was mixed with 1 mL of 1% potassium ferricyanide. The mixture was incubated at 50^{o}c for 20 minutes. After which 1 mL of 10% trichloroacetic acid was added and the resulting mixture centrifuged at 650 rpm for 10 minutes. The mixture (4 mL) was then mixed with 4 mL of de-ionised water and 1 mL of 0.1% ferric chloride and the absorbance was measured at 700 nm. A similar procedure was repeated with methanol as blank, which served as control. Higher absorbance indicates higher reducing power. The assays were carried out in triplicates and the results were expressed as mean values ± standard deviations.

2.11. Nitric oxide (NO) Scavenging Assay

Nitric oxide generated from sodium nitroprusside (SNP) was measured according to the modified method of Marcocci *et al*. [9]. Three milliliters (3 mL) of SNP in phosphate buffered saline (pH 7.4) was added to 2 mL of different concentrations of leaf extract and fractions of *Solanum anomalum* and ascorbic acid (20, 40, 60, 80, and 100 µg/mL) the resulting solutions was then incubated at 25^{o}c for 60 minutes. A similar procedure was repeated with methanol as blank, which served as control. To 3 mL of the incubated sample, 5 mL of Griess reagent (1% sulphonamide in 2 % phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride) was added. The absorbance of chromophore (purple azo dye) that were formed during the diazotisation of nitrite ions with sulphanilamide and subsequent coupling with naphthyl ethylenediamine dihydrochloride was measured at 540 nm. The assays were carried out in triplicates and the results were expressed as mean values ± standard deviations [10]. The percentage inhibition was calculated according to the following equation [11].

%inhibition=$(1-A_1/A_o) \times 100$. Where A_1 = Absorbance of the extract or standard. A_o=absorbance of the control

2.12. Determination of Total Phenolic Content

Total phenolic contents of the crude extract and fractions of the fruits 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 Na_2CO_3 (7%). The resulting mixture was then vortexed for 15 seconds incubated at 40^{o}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 [12, 13].

2.13. Determination of Total Flavonoid Content

The total flavonoids content was estimated using the procedure described by Madhu, *et al.* [13]. 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 5 mL 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.

2.14. Gas Chromatography-Mass Spectrometry Analysis

Gas chromatography-mass spectrometry (GC-MS) data of the active fractions (ethyl acetate) were recorded on an Agilent 7890A gas chromatograph connected with an Agilent MS model 5975C MSD detector (Agilent Technologies, USA). A HP5-MS column 5% phenyl-methylpolysiloxane, 30m × 0.25mm × 0.25µm was used with a helium gas flow under a pressure of 10 psi. The injector temperature was set at 280°C. The oven temperature started from 150°C for 3min, and increased to 300°C at 10°C/min, and held for 5 min at 300°C. The mass spectrometer was operated using the electron ionization mode at 70eV [3, 6]. The phytochemicals were identified by comparison of spectra in the NIST 2011 database.

2.15. Statistical analysis

Data obtained from this work were analyzed statistically using ANOVA (One-way) followed by a post test (Tukey-kramer multiple comparison test). Differences between means were considered significant at 5% level of significance ($p \leq 0.05$).

3. Results

3.1. Genotoxic activity

3.2. Physicochemical Characterization

The effect of *S. anomalum* fruit extract on levels of the physicochemical parameters (root number and root length) are presented in Table 1. This result show that all tested concentrations of *S. anomalum* fruit 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 fruit extract. The average root length in negative and positive control (methotrexate) groups were 5.48±0.27 and 0.26±0.16 cm respectively. However, average root length in 10 mg/mL treatment group was decreased significantly compared to that of the negative control; 0.68±0.07 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 almost normal during the negative control treatment, but at 2.5 mg/mL of *S. anomalum* fruit extract, the roots appeared slightly yellow and at 5 and 10 mg/mL of *S. anomalum* fruit extract, the roots had brownish tips. (Table 1).

3.3. Cytogenetic Analysis.

Table 2 shows the effects of *Solanum anomalum* fruit 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 fruit extract of *S. anomalum* at 10 mg/mL had mitotic index of 16.80±6.93 as compared to 64.80 ± 8.65 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. anomalum* fruit 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 especially in the highest concentration (Table 3) (Figures 1(a). This was significant ($p<0.05$) when compared to negative control group. Fragments or clastogenic breaks of chromosomes were observed to increase with all concentrations of fruit extract (Table 3; Figures 1 (b) and Figures 1(i)). Sticky metaphase were also observed (Figures 1(h) 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. anomalum* had high numbers of cells with micronuclei in the test compared to negative control, which were statistically significant ($p< .05$) (Figures 1 (e)). In addition, cells with membrane damage (Figures 1 (b,c,d,f and i)), binucleated cells (Figures 1(e and h)), and nucleus damage (Figures 1(b,c, d,f,) and 1(i)) were found in various frequencies. Also, apoptotic cells (Figures 1 (e)) were detected in the group treated with the fruit extract.

3.4. Effect of extract and fractions on DPPH free Radicals

The fruit extract and fractions of *S. anomalum* were able to scavenge DPPH free radical via hydrogen donating activity at different concentrations. The scavenging activity increased in a concentration dependent fashion. However, ethyl acetate fraction had the highest inhibitory activity though not compared to control (ascorbic acid) (Figure 2).

3.5. Effect of Fruit Extract and Fractions on Reducing Power Assay

The extract and fractions demonstrated their potential to reduce Fe^{3+} to Fe^{2+}. The result showed that the reducing power of the fruit extract and fractions were concentration dependent. The n-hexane fraction followed by dichloromethane fraction had the highest reducing capacity and their effects were greater than that of the standard drug, ascorbic acid (Figure 3).

3.6. Effect of Fruit Extract and Fraction on Nitric Oxide Assay

The extract and fraction considerably reduced generation of nitric oxide. The crude extract exhibited the highest NO scavenging ability followed by methanol fraction. The activities of the crude extract and methanol fraction were not comparable to that of the standard drug, ascorbic acid. (Figure 4).

3.7. Total Flavonoids Content of *S. anomalum* Extract and Fractions

The result of total flavonoid content of fruit extracts and fractions of *Solanum anomalum* presented in (Figure 5) shows that the dichloromethane fraction contain the highest quantity of flavonoid followed by ethyl acetate fraction.

3.8. Total Phenolic Content of *S. anomalum* Fruit Extract and Fractions

The total phenolic content of the crude extract and fractions as deduced from the calibration graph. The crude extract was found to have the highest phenolic content (105.31 mg/g) followed by methanol fraction (73.97 mg/g) (Figure 6).

3.9. GCMS Analyses of n-Hexane, Dichloromethane and Ethyl Acetate Fractions

The results of GCMS analysis of *n*-hexane, dichloromethane and ethyl acetate fractions show that the fraction contain various pharmacologically active compounds such as (E)-9-Octadecenoic acid ethyl ester, Tetradecanoic acid, Octadecanoic acid, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, myristic acid, palmitoleic acid, (E)-9-Octadecenoic acid ethyl ester , 11-Octadecenoic acid, methyl ester, 9-Octadecenoic acid, (E)-, Octadecanoic acid, Heptadecanoic acid, Cyclodisilazane-2,2,4,4-tetramine, N,N,N',N'-tetramethyl-1,3-bis [tris(methylamino)silyl]-,2(3H)-Furanone, dihydro-5-tetradecyl-, Succinic acid, 2-(3-nitrophenyl) ethyl nonyl ester, 5-Thiazole ethanol, 4-methyl-, Cyclotrisiloxane, hexamethyl, Tridecanoic acid, 12-methyl-, methyl ester, Tetradecanoic acid, Octadecanoic acid, Piperidine, 1-acetyl- among others (Tables 4, 5 and 6).

Table-1. Cytotoxicity of *Solanum anomalum* fruit extract on growing roots of Onion (*Allium cepa*)

Treatment group	Concentration of extract (mg/mL)	Average root Number ± S.D	Average root length (cm)± S.D
Negative control	Tap water	36.21±5.14	5.48±0.27
Methotrexate	0.1	10.55±5.28[a]	0.28±0.16 [a]
Solanum anomalum	2.5	27.26±4.28[a]	2.08±0.13[a]
	5.0	22.30±3.83	0.90±0.05[a]
	10.0	18.54±3.48[a]	0.68±0.07[a]

Values are expressed as mean ±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 (%)± S.E
Negative control	Tap water	500	324	64.80±8.65
Methotrexate	0.1	500	18	3.60±0.25[a]
Solanum anomalum	2.5	500	296	59.20±4.72
	5.0	500	202	40.44±3.88[a]
	10.0	500	84	16.80±6.93[a]

Values are expressed as mean ±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 of fruit 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	-	0.18±0.02	0.14±0.06	2.12±0.14	-
Methotrexate	0.10	2.23±1.06 [a]	19.36±4.29[a]	15.10±3.16[a]	48.68±5.88[a]	2.84±0.38 [a]
Solanum anomalum	2.5	1.16±0.33[a]	4.24±1.12 [a]	1.44±0.45[a]	15.20±4.28[a]	10.25±1.46[a]
	5.0	3.23±0.86[a]	6.55±3.28[a]	1.53±3.26[a]	22.87±5.28[a]	2.14±0.73[a]
	10.0	2.56±0.25[a]	8.63±2.66	4.42±0.44[a]	32.25±5.84	1.34±0.35[a]

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

Table-4. GCMS analysis of n-hexane fraction of *S. anomalum* fruits

PEAK	RT	COMPOUND NAME	FORMULA	MOL. MASS
1.	2.799	Cycloeicosane	$C_{20}H_{40}$	280.313
2.	3.022	2(3H)-Furanone, dihydro-5-tetradecyl-	$C_{18}H_{34}O_2$	282.255
3.	3.136	Succinic acid, 2-(3-nitrophenyl)ethyl nonyl ester	$C_{21}H_{31}NO_6$	393.215
4.	3.930	5-Thiazole ethanol, 4-methyl-	C_6H_9NOS	143.040
5.	3.993	Cyclotrisiloxane, hexamethyl	$C_6H_{18}O_3Si_3$	222.056
6.	6.502	Tridecanoic acid, 12-methyl-, methyl ester	$C_{15}H_{30}O_2$	242.224
7.	6.777	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.208
8.	7.331	Octadecanoic acid	$C_{18}H_{36}O_2$	284.271
9.	7.428	Piperidine, 1-acetyl-	$C_7H_{13}NO$	127.099
10.	7.514	Propanenitrile, 3-(1-azepanyl)-3-oxo-	$C_9H_{14}N_2O$	166.110
11	7.617	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.255
12	7.783	Palmitoleic acid	$C_{16}H_{30}O_2$	254.224
13	7.868	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.240
14	8.143	Z,Z-6,27-Hexatriactontadien-2-one	$C_{36}H_{68}O$	516.527
15	8.383	Heptadecanoic acid	$C_{14}H_{28}O_2$	228.208
16	8.514	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.271
17	8.543	(E)-9-Octadecenoic acid ethyl ester	$C_{18}H_{34}O_2$	282.255
18	8.634	Methyl stearate	$C_{19}H_{38}O_2$	298.287
19.	8.760	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282.255
20	8.863	Octadecanoic acid	$C_{18}H_{36}O_2$	284.271
21	9.246	Tungsten, tris(.eta.-3-allyl)-1-butyl imido) propyl)	$C_{16}H_{29}N$	419.180
22	9.463	Octodrine	$C_8H_{19}N$	129.151
23	9.686	Cyclodisilazane-2,2,4,4-tetramine,N,N,N',N'-tetramethyl-1,3-bis[tris(methylamino)silyl]-	$C_{10}H_{40}N_{12}Si_4$	440.257
24	10.263	4-Cyclopentylaminomethylene-2-(4-fluoro-phenyl)-5-propyl-2,4-dihydro -pyrazol-3-one		
25	11.343	Morphinan-6,14-diol, 7,8-didehydro-4,5-epoxy-3-methoxy-17-methyl-, (5.alpha.,6.alpha.)-	$C_{18}H_{21}NO_4$	315.147

Table-5. GCMS analysis of dichloromethane fraction of *S. anomalum* fruits

PEAK	RT	COMPOUND NAME	FORMULA	MOL. MASS
1.	2.816	Phenylethyl alcohol	$C_8H_{10}O$	122.073
2.	3.136	Phthalic acid, butyl ester, ester with butyl glycolate	$C_{18}H_{24}O_6$	336.157
3.	3.913	5-Thiazole ethanol, 4-methyl-	C_6H_9NOS	143.040
4.	5.096	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	$C_7H_{10}O_2$	126.068
5.	6.474	Tetradecanoic acid, methyl ester	$C_{15}H_{30}O_2$	242.224
6.	6.742	N-[4-(Chloro-difluoro-methoxy)-phenyl]-2-piperidin-1-yl-acetamide	$C_{14}H_{17}ClF_2N_2O_2$	318.094
7.	7.480	E-11-Tetradecenoic acid	$C_{14}H_{26}O_2$	226.193
8.	7.588	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.255
9.	7.743	Palmitoleic acid	$C_{16}H_{30}O_2$	254.224
10.	7.834	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.240
11	8.348	1,1'-Bicyclohexyl, 4-methyl-4'-propyl-	$C_{17}H_{34}O_2$	270.255
12	8.486	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296.271
13	8.606	Methyl stearate	$C_{19}H_{38}O_2$	298.287
14	8.726	Oleic acid	$C_{18}H_{34}O_2$	282.255

15	8.829	Octadecanoic acid	$C_{18}H_{36}O_2$	284.271
16	9.434	2-Pyrimidinamine, 4-[4-[3-[1-(2-hydroxyethyl) piperidin-4-yl]propyl] piperidino]-6-methyl-N-(5,6-dichlor o-1,3(1H)-benzimidazol-2-yl)-	$C_{27}H_{37}C_{l2}N_7O$	545.243
17	9.674	Protocatechoic acid,3TBDMS derivative	$C_{25}H_{48}O_4Si_3$	496.286
18	12.424	2,6-Dihydroxybenzoic acid, 3TBDMS derivative/1-Hexacosanol,	$C_{25}H_{48}O_4Si_3$	496.286
19.	12.618	Androsta[17-16-b]furan-5'-imine, 4 '-methylene-3-methoxy-N-cyclohexyl	$C_{29}H_{45}NO_2$	439.345
20	14.973	2,5-Dihydroxybenzoic acid, 3TBDMS derivative	$C_{25}H_{48}O_4Si_3$	496.286
21	15.510	Cyclodisilazane-2,2,4,4-tetramine, N,N,N',N'-tetramethyl-1,3-bis[tris (methylamino)silyl]-	$C_{10}H_{40}N_{12}Si_4$	440.257
22	16.138	3,5-Dihydroxybenzoic acid, 3TBDMS derivative	$C_{25}H_{48}O_4Si_3$	496.286
23	17.459	2,6-Dihydroxybenzoic acid, 3TBDMS derivative	$C_{25}H_{48}O_4Si_3$	496.286
24	17.584	Androsta[17-16-b]furan-5'-imine, 4 '-methylene-3-methoxy-N-cyclohexyl	$C_{29}H_{45}NO_2$	439.345
25	17.904	5Alpha-cyano-3 -methoxymethylenecholestane, (E)-	$C_{30}H_{49}NO$	439.381

Table-6. GCMS analysis of ethyl acetate fraction of *S. anomalum* fruits

PEAK	RT	COMPOUND NAME	FORMULA	MOL. MASS
1.	3.616	Methenamine	$C_6H_{12}N_4$	140.106
2.	6.468	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242.224
3.	6.754	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.240
4.	7.120	Diphenylacetylene	$C_{14}H_{10}$	178.078
5.	7.480	13-Borabicyclo[7.3.0] tridecane, 13-propoxy-, (Z)- or (E)-	$C_{15}H_{29}BO$	236.231
6.	7.583	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.255
7.	7.748	Oleic acid	$C_{18}H_{34}O_2$	282.255
8.	7.828	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.240
9.	8.480	9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	296.271
10.	8.600	Methyl stearate	$C_{19}H_{38}O_2$	298.28718
11	8.726	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282.25588
12	8.829	Octadecanoic acid	$C_{18}H_{36}O_2$	284.27153
13	8.949	4-(Adamantan-1-yl)-1H-pyrazole	$C_{13}H_{18}N_2$	202.146
14	9.434	Cyclodisilazane-2,2,4,4-tetramine, N,N,N',N'-tetramethyl-1,3-bis[tris (methylamino)silyl]-	$C_{10}H_{40}N_{12}Si_4$	440.257
15	9.652	Silane, (hexacosyloxy)trimethyl-	$C_{29}H_{62}OSi$	454.456
16	10.297	Acetamide, 2,2,2-trifluoro-N-(5,6, 7,9-tetrahydro-2-hydroxy-1,3,10-trimethoxy-9-oxobenzo(a)heptalen-7-yl)-, (S)-	$C_{21}H_{20}F_3NO_6$	439.124
17	10.429	5Alpha-cyano-3-ethoxymethylenecholestane, (E)-	$C_{30}H_{49}NO$	439.381
18	10.680	2,5-Dihydroxybenzoic acid, 3TBDMS derivative	$C_{25}H_{48}O_4Si_3$	496.286
19.	11.469	.pi.-Pentamethylcyclopentadienyl-di (ethylthio)-diethylaminocarbin-wolfram(vi)	$C_{19}H_{35}NS_2W$	525.172
20	12.338	tert-Butyl(dimethyl)silyl 2,5-bispyrrol [tertbutyl(dimethyl)silyl]oxymorphobenzoate	$C_{25}H_{48}O_4Si_3$	496.286
21	12.864	2,3-Dihydroxybenzoic acid, 3TBDMS derivative	$C_{25}H_{48}O4Si_3$	496.286
22	14.527	Androsta[17-16-b]furan-5'-imine, 4'-methylene-3-methoxy-N-cyclohexyl	$C_{29}H_{45}NO_2$	439.345
23	14.978	Colchiceine, N-desacetyl-N-TFA-	$C_{21}H_{20}F_3NO_6$	439.1241
24	15.047	3,5-Dihydroxybenzoic acid, 3TBDMS derivative	$C_{25}H_{48}O_4Si_3$	496.286
25	15.396	2,6-Dihydroxybenzoic acid, 3TBDMS derivative	$C_{25}H_{48}O_4Si_3$	496.286
26	16.327	5-(4-Chlorophenyl)-6-ethylpyrimidine-2,4-diamine, N,N'-bis(trifluoro acetyl)-	$C_{16}H_{11}ClF_6N_4O_2$	440.047
27	17.253	Silane, diethyl(4-chlorobenzyloxy) hexadecyloxy-	$C_{29}H_{60}O_2Si$	468.436
28	17.756	Silane, (hexacosyloxy) trimethyl-	$C_{29}H_{62}OSi$	454.456
29	17.790	Silane, diethyloctadecyloxy(trans-4-methylcyclohexyloxy)-	$C_{29}H_{60}O_2Si$	468.436

30	17.882	Silane, diethyloctadecyloxy(trans-4-methylcyclohexyloxy)-	$C_{29}H_{60}O_2Si$	468.436

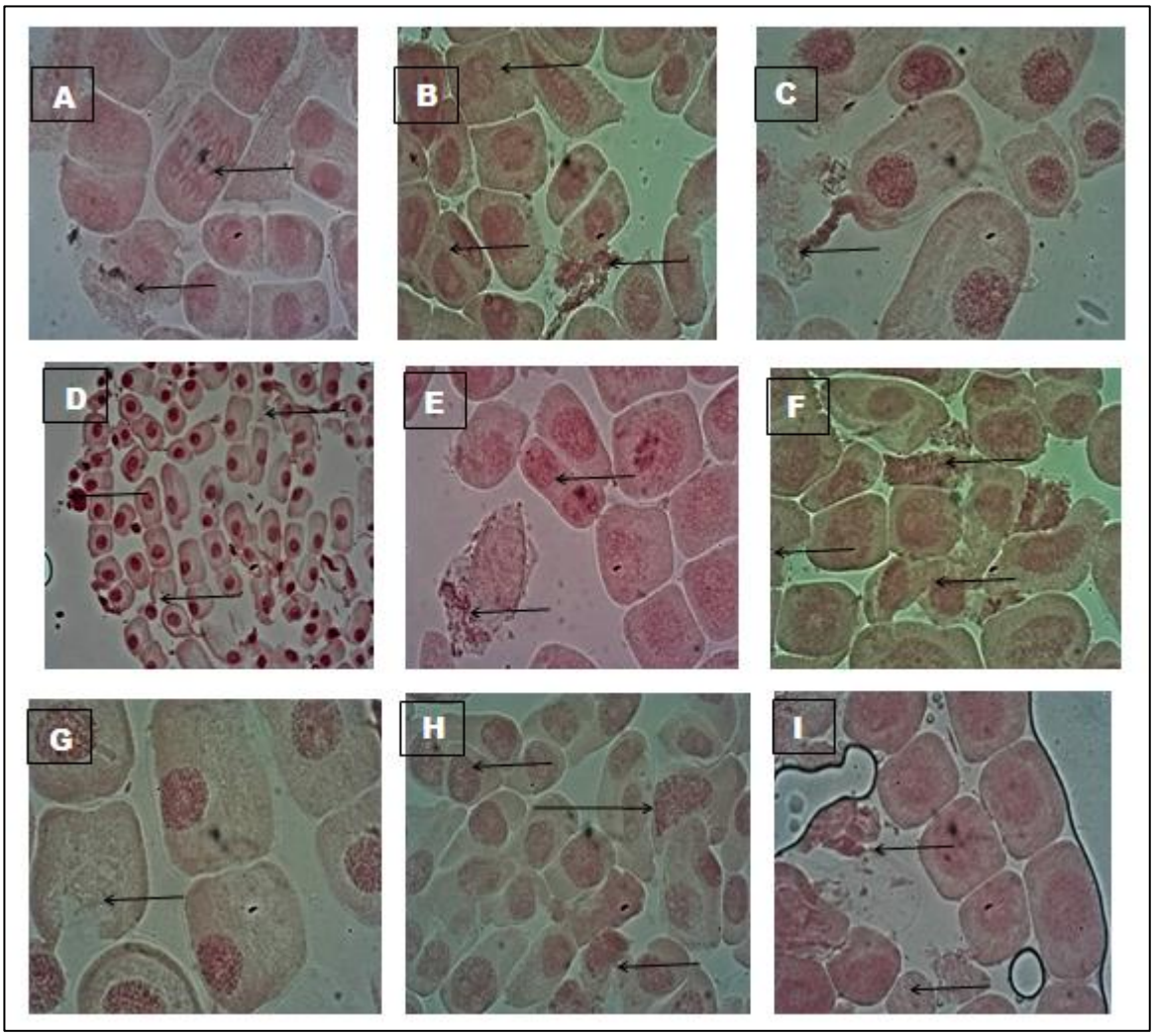

Figure-1. Photomicrograph showing the mitotic and chromosomal aberrations of *Allium cepa* root meristem cells after *Solanum anomalum* fruit extract treatments under light microscope X40 magnification. Arrows indicate (A) Bridge and nuclear damage (B) Chromosomal fragmentation, binucleated cells and nuclear damage (C) nuclear and cell wall damage (D) Nuclear and membrane damage(E) Binucleated cells and apoptotic bodies (F) membrane and nuclear damage (G) dead cells (H) sticky metaphase and binucleated cells (I) Nuclear damage and fragmentation

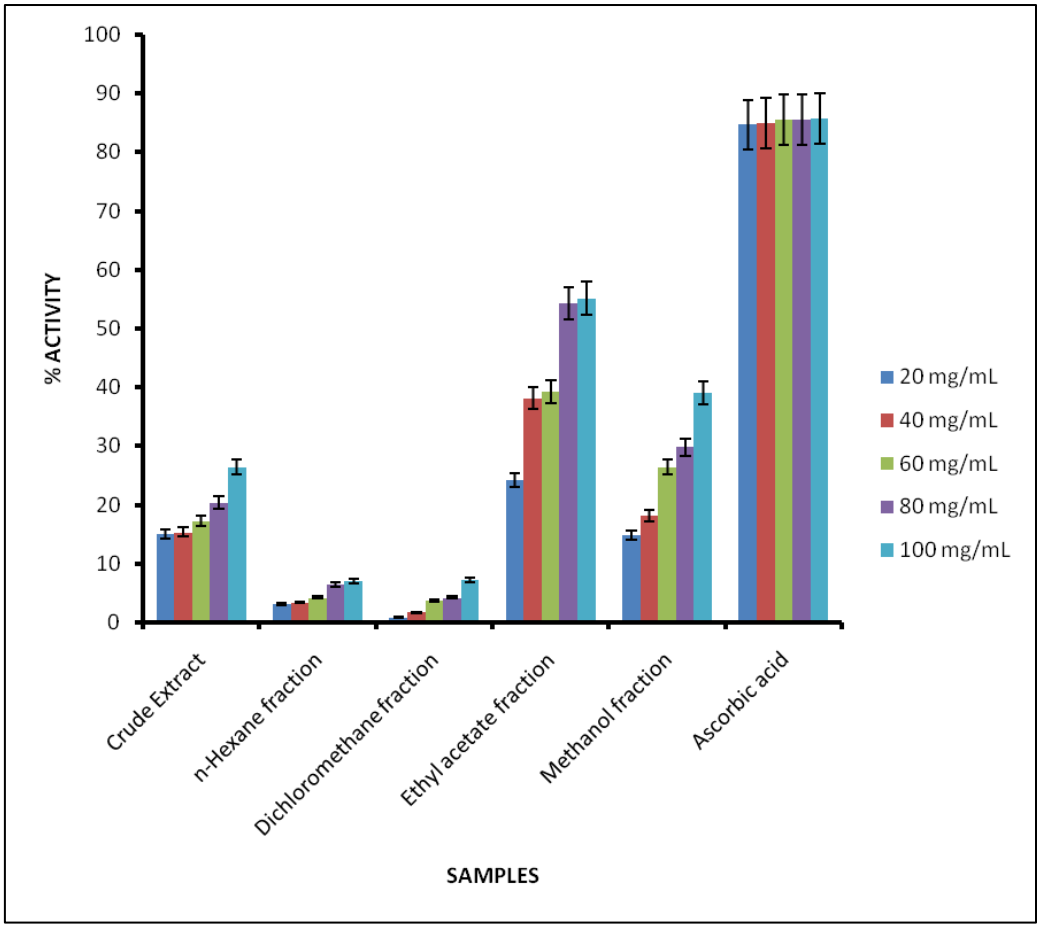

Figure-2. Effect of fruit extract and fractions of *Solanum anomalum* on DPPH

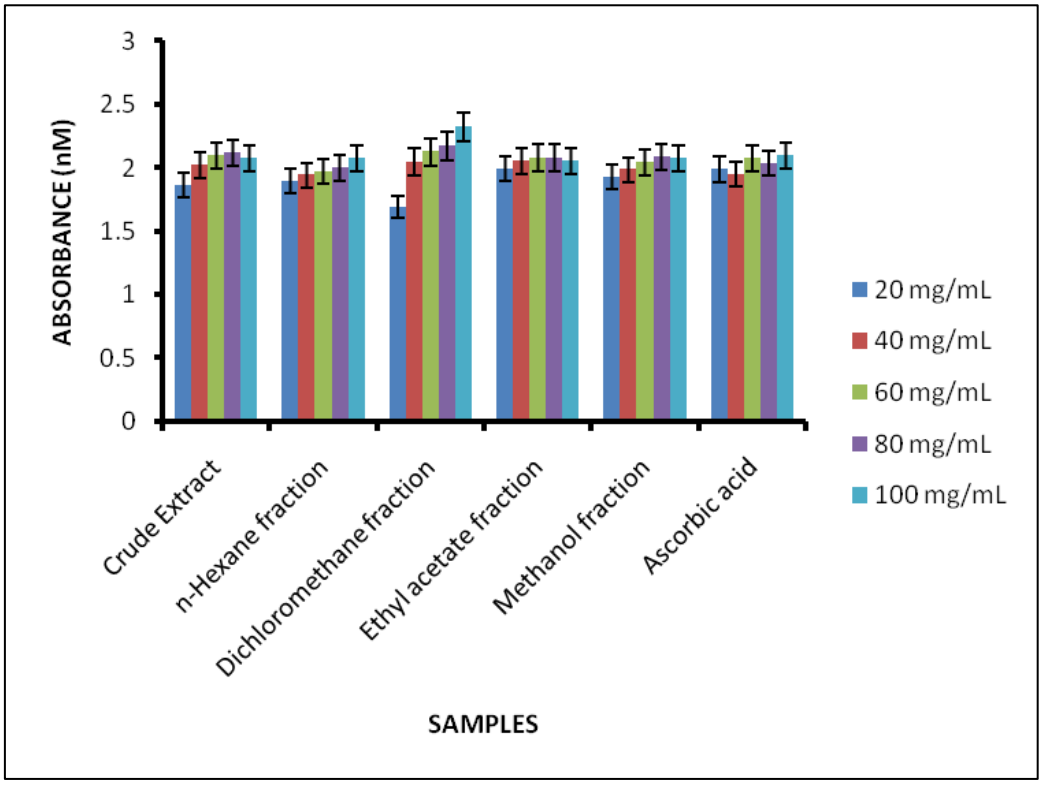

Figure-3. Effect of fruit extract and fractions of *S. anomalum* on FRAP

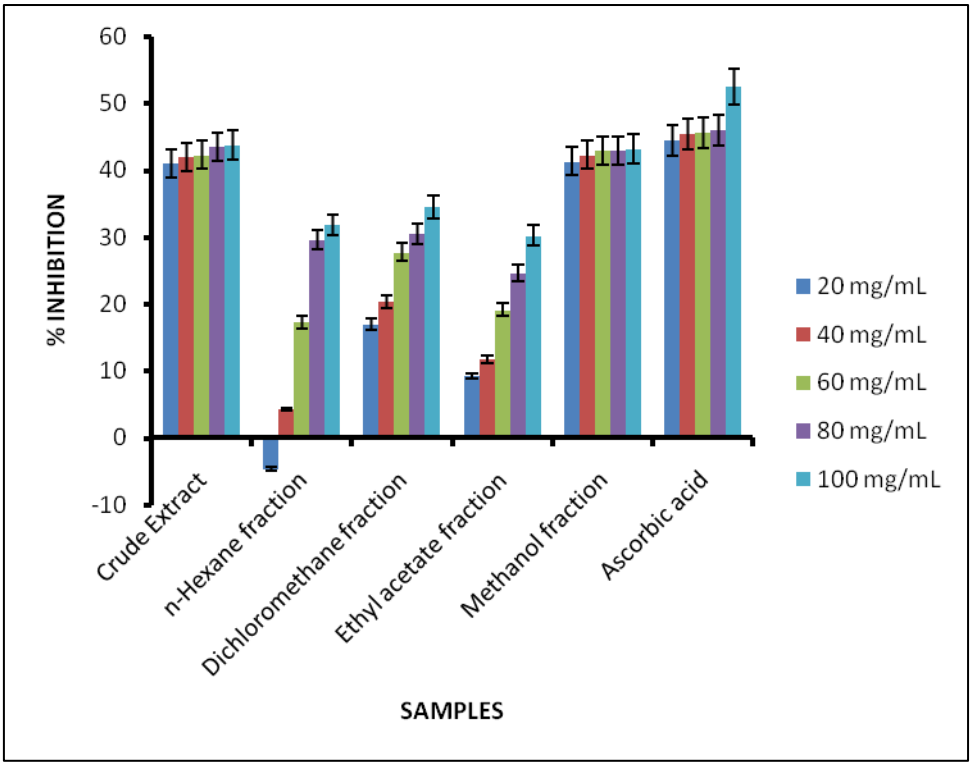

Figure-4. Effect of fruit extract and fractions of *Solanum anomalum* on Nitric oxide

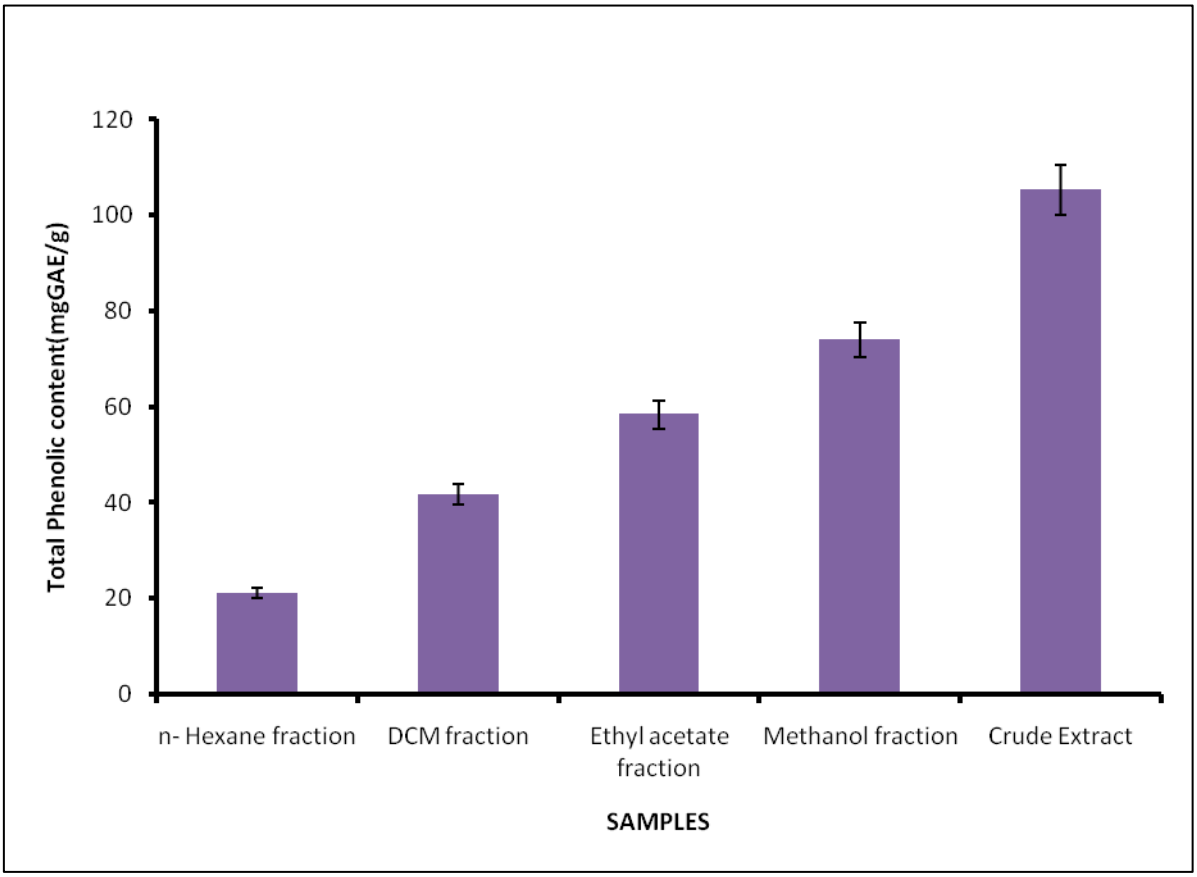

Figure-5. Total phenolic contents of extract and fractions of *S. anomalum*

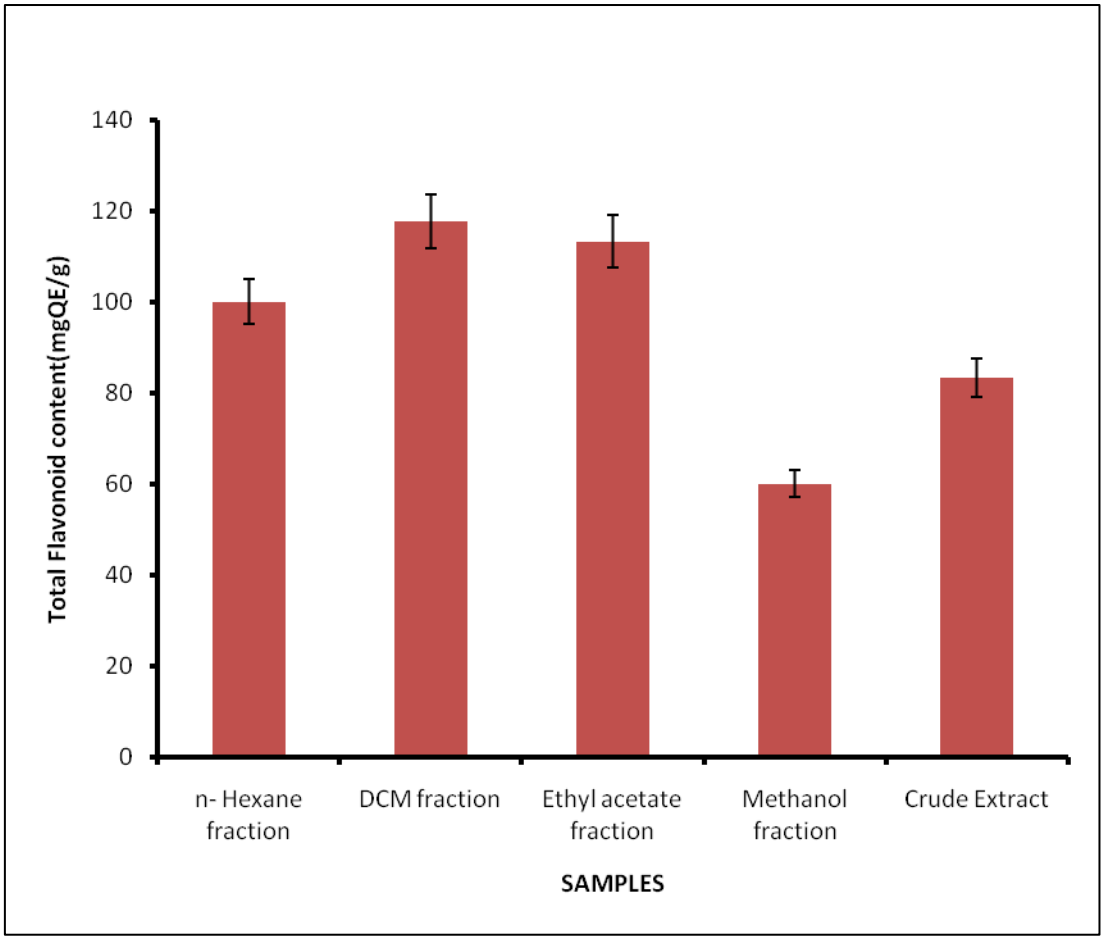

Figure-6. Total flavonoid contents of extract and fractions of *Solanum anomalum*

4. Discussion

In this study, toxic effects of *Solanum anomalum* fruit 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 *Solanum anomalum* fruit extract (59.20 %, 40.44 % and 16.80 % 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 [14], while a decrease below 50% usually has sublethal effects [15] and is called cytotoxic limit value [16]. 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. 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 [17]. Mitodepressive effects of some herbal extracts, including the ability to block the synthesis of DNA and nucleus proteins, were reported earlier [18, 19]. Previous studies on herbal extracts had reported their mitosis arrest potentials [20]. The decreased mitotic index in *A. cepa* roots treated with *S. anomalum* fruit extract is probably due to either disturbances in the cell cycle or chromatin dysfunction induced by extract-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 [21]. 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. 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 [22]. The presence of chromosome fragments is an indication of chromosome breaks, and can be a consequence of anaphase/telophase bridges [23]. 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. Lower concentrations of the fruit extract were found to have increased frequencies of MNC formation. However, reduced MNC frequency at the highest concentration could have been 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 [24, 25]. Previous studies have suggested MNC-induced effect of various plant extracts such as *Lavandula stoechas* and *Ecballium elaterium* [26], *Azadirachta indica* [27] *Psychotria* species [20].

In this study, membrane damage 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 [26]. 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.

The extract and fractions were found to exhibit strong *in vitro* activity in the various models studied. This explained their therapeutic potentials in the treatment of various disease conditions especially in inflammatory conditions and poisoning. The results of this study further show that the extract contains a high phenolic and favonoid content especially the crude extract, dichloromethane and methanol fractions. Flavonoids such as quercetin have been reported to demonstrate mutagenic and genotoxic potentials in various studies [28]. The high phenols and flavonoids contents in the fruit extract must have contributed to the observed cytotoxic and genotoxic activities in this study [29]. We had previously reported antioxidant, genotoxic and cytotoxic activities on the leaf extract and fractions of *Solanum anomalum* [29]. The results of this study corroborate that earlier reported on the leaf of this plant.

5. Conclusion

The results of this study show that the fruit extract of *S. anomalum* has *in vitro* antioxidant potentials, contains some pharmacological active compounds and 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.

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