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Studies on the proximate analysis of *Solanum aethiopicum*, *Lactuca taraxacifolia* and *Talinum triangulare* and potential cytotoxic effects of their aqueous extract using *Allium cepa* assay

[*1]Yekeen, T. A.,[1]Adetiba, O. A., [1]Azeez, M. A., [1]Falodun, M. A., [1]Akintaro, S. I. and [2]Yekeen, T. A.

[1]Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria
[2]Affliate of Department of Pure and Applied Chemistry, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

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ABSTRACT

Sources of vegetables purchased for consumption in communities in Nigeria as well as their level of contamination or bioaccumulation of toxicant is not always known. This study evaluates the proximate analysis of Solanum aethiopicum (L), Lactuca taraxacifolia (Willd) and Talinum triangulare (L) purchased from Sabo market in Ogbomoso, Nigeria. The potential cytotoxic effect of their aqueous extracts was also tested on Allium cepa (L). The proximate analysis revealed that the vegetable contained valuable nutrients that are needed for body development. The protein and moisture contents were significant in S. aethiopicum and T. triangulare respectively. The heavy metal evaluation showed the presence of iron, zinc, lead, and copper ions at various degrees in the vegetable extracts with significant difference observed in all except for lead. The extract of the vegetables showed significant inhibition of A. cepa root length at 10.0, 25.0 and 50.0 ppt of S. aethiopicum, 25.0 and 50.0 ppt of L. taraxacifolia and only 50.0 ppt of T. triangulare compared to control ($P<0.05$), while the EC_{50} values of 5.7, 14.0 and 22.4 ppt which reflected the order of their toxicity were respectively obtained. Variations in the value of Mitotic index and inhibition were obtained with various chromosomal aberrations observed in the extracts at different concentrations. The results revealed that the tested extracts possess inhibitory, and mitodepressive effects on the root growth and cell division, and also induced chromosomal aberrations in A. cepa. The vegetables might have bioaccumulated toxicants which may hitherto affect the consumers.

Key words- Vegetables, *Solanum aethiopicum*, *Lactuca taraxacifolia*, *Talinum triangulare*, *Allium cepa*, Genotoxic, Aberration

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INTRODUCTION

Increased awareness on the usefulness of vegetable inclusions in human food has enhanced their consumption as part of daily diet. Commercially consumed leafy vegetables are those plants whose leaves or aerial parts have been integrated in a community's culture for use as food over a large span of time [1]. They are highly recommended because they have a relatively high nutritional value and their consumption gives diversity to daily food intake, adding flavour and zest to the diet [2]. Vegetables constitute essential components of the diet, by contributing protein, vitamins, iron, calcium and other nutrients which are usually in short supply [3]. Besides these bio-chemicals, the moisture, fiber and ash contents, and the energy values of individual vegetable and plant species have also been regarded of importance to human health [4, 5].

The safety of these vegetables as a source of food has been a major public concern worldwide [6]. During the last decades, the increasing demand for food safety has stimulated research regarding the risk associated with consumption of food stuffs contaminated by pesticides, heavy metals and/or toxins [7] which are produced either naturally as part of the plant or artificially as a result of environmental pollution in areas where these plants are being cultivated [8].

In Ogbomoso, south western part of Nigeria, S*olanum aethiopicum* (L), *Lactuca taraxacifolia* (Willd) and *Talinum triangulare* (L) are examples of some leafy vegetables commonly consumed. In this zone, these vegetables are referred to as 'Gbagba', 'Yanrin' and 'Gbure' respectively. With the exception of *L. taraxacifolia*, these vegetables are produced in large quantity from different sources in order to meet up with the demand of the consumers.

Solanum aethiopicum (family Solanoaceae) is the most popular species of egg plants domesticated from South Asia [9]. It has high levels of phenol and vitamin C. Taylor [10] reported the protein and soluble oxalate content of *S. aethiopicum* to be significantly higher than *S macrocarpon* while the fruits from both species were reported to have high quality protein with more amino acid concentration in 15 out of 17 amino acids determined in the latter [11].

Talinum triangulare (family Portulacaceae) originated from tropical Africa, now is widely cultivated as a medicinal and food crop in West Africa (especially in Nigeria), Asia, and South America. It has been reported to have anti-oxidant property [12, 13] and also used in the treatment of diseases.

Lactuca taraxacifolia (family Asteraceae) which initially called *Launaea taraxacifolia* is not very common compared with *S. aethiopicum* and *T. triangulare*. It has been observed to be a good source of minerals like phosphorous and iron and also contain some protein. The best time of harvest appear to be between 6th and 8th week after planting [14]. Like others, it has medicinal properties. Obi *et al.* [15] reported that the consumption of *L. taraxacifolia* could to a large extent prevent infection or further replication of the measles virus. In animal, evaluation has shown that it has cholesterol lowering effect [16].

These plants had been known for their nutritional quality for long. However, the source of their production is not always known to the final consumers who purchase the vegetables from the market. Some are grown on poultry droppings, contaminated soil etc., through which the plants might have bioaccumulated some toxicants. Report has shown that vegetables can absorb metals from the soil as well as from deposits on parts of the vegetables exposed to the air from polluted environments [17, 18]. This research therefore sought to evaluate the nutritional quality of the aforementioned vegetables obtained from an open market and also tests the potential cytotoxic effects of their aqueous extracts using *Allium cepa* assay.

MATERIALS AND METHODS

Collection of samples: Fresh leaves of *S. aethiopicum* (L), *L. taraxacifolia* (Willd) and *T. triangulare* (L) were obtained from the Sabo market, Ogbomoso and were identified and assigned voucher number LHO 205, LHO 208 and LHO 209, respectively at the herbarium of Department of Pure and Applied Biology, LAUTECH, Ogbomoso, Nigeria.

Preparation of extracts: The leafy part (500 g) of each of the vegetables was boiled separately in 1litre of water at 100°C for 30 minutes. The resultant extract was sieved and used for further analysis.

Proximate analysis of the vegetables: Air dried fresh leaves (10g) were used for evaluation of moisture, protein, fat, ash and crude fibre based on the standard method [19] while carbohydrate was calculated by percentage differences.

Heavy metal analysis: In order to simulate the pattern of vegetable consumption, heavy metals were evaluated from the aqueous extract of the vegetable. The solution was finally boiled with 1:5 mixtures of concentrate acids; HCl and HNO_3, in order to digest all organic matters, and then filtered after cooling. Samples were analysed using Atomic Absorption Spectrophotometer (Buck 210 AAS, 2005, USA). The water used as diluents and control was also analysed for its physical and chemical properties.

Allium cepa **assay**: The modified *Allium cepa* (L) assay was used with Fiskesjo [20], Rank and Neilson [21] and Bakare *et al*. [22] as templates. The toxic potential of the vegetable extracts was evaluated using onion bulbs obtained at the Sabo market, Ogbomoso, Oyo state. The onion bulbs were sun dried for 3 weeks and the dried outer scales were removed carefully leaving the ring of root primordial exposed and intact. Seven concentrations (0.5, 1.0, 2.5, 5.0, 10.0, 25.0 and 50.0 ppt) of each vegetable extract were prepared from their respective stock solution with well-water used as diluents and control. Ten onion bulbs were planted per concentration at room temperature (25±1°C) in the dark. The extract per bottle was changed every 24 h and root tips from selected bulbs were harvested at 48 h for microscopic and 72 h for macroscopic evaluations as previously described by Lateef *et al*.[23].

Statistical analysis: SPSS version 15 was used for data analysis. Student T-test was used to compare the means of each treated onion bulb per concentration from each of the vegetables with that of the control. One – way ANOVA and Duncan test were used to compare the means of the dividing cells counted per concentration. Level of significance were considered at $P<0.05$.

RESULTS

Physicochemical analysis of the water sample used as control and diluents is shown in table 1. Compared with the maximum concentration level (MCL) of WHO [24], all parameters examined were in conformity except the value obtained for lead. However Chromium, Copper and Cadmium ions were not detected in the water sample.

Proximate analysis of the vegetables showed variation in the level of protein, ash, moisture, fat, fibre and carbohydrate (Table 2). Significant difference ($P<0.05$) was observed only in protein and moisture contents with highest values obtained for *S. aethiopicum* and *T. triangulare*, respectively. Least values of ash and fibre were found in *S. aethiopicum* compared to the others, while the carbohydrate was similar in all the evaluated vegetables with least value obtained in *T. triangulare.* Variation was observed in the heavy metal content of the aqueous extracts of the three vegetables with significant difference observed only in Iron, zinc and magnesium ions. *L. taraxacifolia* had highest value of iron while there was no difference in the iron content of *S. aethiopicum* and *T. trangulare*. The least Zinc value was obtained in the *T. triangulare* while there was no difference in the value obtained for other two vegetables. Chromium and cadmium ions were not detected in the three vegetables, while copper was not detected only in *T. triangulare*.

Macroscopic analysis: Table 3 shows the growth response of *A. cepa* root to extracts of *S. aethiopicum*, *L. taraxacifolia* and *T. triangulare*. There was dose dependent reduction in the mean root length (MRL) from 0.5 ppt to 50.0 ppt in both *S. aethiopicum* and *T. triangulare.* Highest MRL value was obtained at 1.0 ppt of *L. taraxacifolia*. The MRL values of the treated roots were significantly different ($P<0.05$) from control at 10.0, 25.0 and 50.0 ppt of *S. aethiopicum*, 25.0 and 50.0 ppt of *L. taraxacifolia* and only 50.0 ppt of *T. triangulare* ($P<0.05$). The EC_{50} values of 5.7, 14.0 and 22.4 ppt were respectively obtained for *S. aethiopicum, L. taraxacifolia* and *T. triangulare* (Figure 1).

Microscopic analysis: The cytotoxic effects of the leaf extracts of *S. aethiopicum, L. taraxacifolia* and *T. triangulare* on *A. cepa* root cell is shown in Tables 4, 5 and 6, respectively. The numbers of dividing cells obtained for *A. cepa* treated with the extract of *S. aethiopicum* were lower than that of the control except at 2.5 ppt. Significant reduction in the dividing cells was observed at 10.0, 25.0 and 50.0 ppt similar to the result of macroscopic evaluation. Unlike in other extracts, complete cell arrest was observed in almost all slide prepared from 50 ppt of *S. aethiopicum* with the only dividing cell obtained happened to be an aberrant cell. In *L. taraxacifolia* treated onions, the number of dividing cells at all concentrations were lower compared to control with significant difference ($p<0.05$) observed only at 25.0 ppt. However, there was a significant difference in the number of dividing cells at 5.0 and 25.0 ppt of *A. cepa* treated with extract of *T. triangulare*. This deviates from what was observed in the root length where only 50 ppt showed significant difference. The mitotic index values obtained with the three extracts showed that cell division was inhibited more prominently with *S. aethiopicum***.** Chromosomal aberrations were observed but not at all concentrations of each of the leaf extracts while none was observed in the control (Table 4, 5 and 6). The aberration observed include c- mitosis, chromosome bridge, sticky chromosome and fragmented chromosome as shown in Figure 2 a, b, c and d respectively.

DISCUSSION

Proximate analysis established that the vegetables contained high level of nutritive elements. The results obtained were relatively high compared to the previous studies reported for proximate analysis in most of the parameters [14, 25]. The fibre content obtained was relatively high which provides advantage on the consumption of the vegetables. High fibre rich food usually produces loose stool, reduces stool transit time in the intestine and discourages colon cancer [26]. Some metals like copper and zinc are essential for important biochemical and physiological functions and necessary for maintaining health throughout life [27, 28, 29], but high value of heavy metals like Pb, in the vegetable is worrisome. Although the water used as control has elevated Pb value, the value obtained from the leaf extracts were higher which shows that the leaves have additional heavy metal apart from those in the water. The toxic effects of vegetable extracts as observed in this study could have resulted from the individual or synergistic effects of these metals ions. Macroscopic evaluation showed increased inhibition of root growth with increasing concentration in most cases. The EC_{50} values revealed the order of toxicity of the extracts in terms of *A. cepa* root inhibition to be *S .aethiopicum*> *L. taraxacifolia*> *T. triangulare.* This was consistent with the result obtained for microscopic evaluation except that there was more inhibition in the *T. triangulare* compared to *L. taraxacifolia*. The value of mitotic index and inhibition confirmed that the extracts were toxic to the *A. cepa* root cells in the three vegetables. The suppression of mitotic activity was often used in tracing cytotoxicity [30] which is usually accompanied by an increase in cells with abnormal chromosome orientation [31]. A decrease in the mitotic index also indicates that the root growth medium used interfered with the normal sequences of mitosis; such a reduction in mitotic activities could be due to inhibition of DNA synthesis [32]. The order of toxicity as obtained from the macroscopic evaluation is adhered to since any genotoxic effect manifested in a test sample; either directly or indirectly, is likely to result in inhibition of growth [33]. The highest toxic effect observed in *A. aethiopicum* reflected from the root inhibition and induction of cell arrest at its highest concentration compared to control and other extracts. The individual metal ion (Pb, Cu and Zn) as observed in the extracts had been implicated to have cytotoxic effects. Lerda [34] reported Pb to be mutagenic while Cu, Zn, (Mn and NH3, not evaluated in this study) were report in several studies to have cytotoxic effects and implicated in inhibition of growth of *A. cepa* [33, 35].

Table 1: Physicochemical properties of the Water used and WHO's standard

Parameters	Value	MCL
Physiochemical		
pH	7.38(±0.05)	-
DO	7.45(±0.61)	-
TSS	141(7.21)	-
TDS	87 (6.21)	-
SO_4	36.24 (±1.56)	500
NO_3	38.251(±1.32)	50
Salinity	0.08(±0.02)	-
Conductivity (cm)	174(±0.82)	250
BOD	8.7(±0.47)	-
Alkalinity	50(±2.15)	10-500
Heavy metals		
Fe	0.24 (±0.02)	2
Zn	0.81(±0.23)	3
Pb	0.02(±0.01)	0.01
Cr	ND	0.05
Cd	ND	0.03
Cu	ND	2

DO- Dissolved oxygen, TSS- Total suspended solid, TDS- Total dissolved solid, - Nitrate, BOD- Bio-oxygen demand, - Sulphate, MCL- Maximum concentration level WHO [24]
-Measurement were in mg/L (ppm) except otherwise stated
- Values in parenthesis represent standard error of mean obtained from three replicates

Lead is among the most abundant heavy metals and is particularly toxic [36]. Zinc has been reported to cause single break in DNA [37] and chromosomal aberrations [38] while Cd, Cu and Fe were reported to give reactive oxygen species in eukaryotic systems [39, 40]. Although, source(s) of the metal contents in these vegetables cannot be pin pointed or traced specifically, thus, considered as limitation of this study. Nevertheless, the results obtained showed that the tested extracts exhibit inhibitory and mitodepressive effects on the root growth and cell division, and also induce chromosomal aberration in *A. cepa* cells.

This study therefore, revealed that the vegetables obtained from the market might have bioaccumulated toxicant which may hitherto affect the well-being of the consumer.

Table 2: Proximate and heavy metal analysis of *S. aethiopicum*, *L. taraxacifolia* and *T. triangulare*

	S. aethiopicum	*L. taraxacifolia*	*T. triangulare*
Proximate analysis (%) from leaves			
Protein	29.87^{a}(±2.12)	19.44^{b}(±1.46)	15.85^{b} (±1.56)
Ash	10.14 (±1.65)	20.34 (±1.74)	23.74 (±1.20)
Moisture	13.09^{b} (±1.42)	7.44^{c}(±0.08)	17.47^{a} (±1.46)
Fat	0.57 (±0.07)	0.49(±0.05)	0.44 (±0.02)
Fiber	9.23 (±1.80)	14.39 (±1.89)	9.42 (±0.91)
CHO	37.10(±4.72)	37.90 (±3.86)	33.08 (±3.23)
Heavy metal analysis (ppm/mg/l)of aqueous extracts			
Fe	0.80^{b}(±0.03)	1.84$^{a(}$±0.01)	0.37^{b}(±0.01)
Zn	0.99^{a} (±0.01)	0.95^{a}(±0.17)	0.05^{b}(±0.06)
Pb	0.04± (0.01)	0.07(±0.02)	0.05(±0.01)
Cd	ND	ND	ND
Cr	ND	ND	ND
Cu	0.09^{b}(±0.02)	0.24^{a} (±0.02)	0

CHO- Carbohydrate, ND- Not detected. N=3
Mean value with different alphabets were significant based on Duncan test (P<0.05)

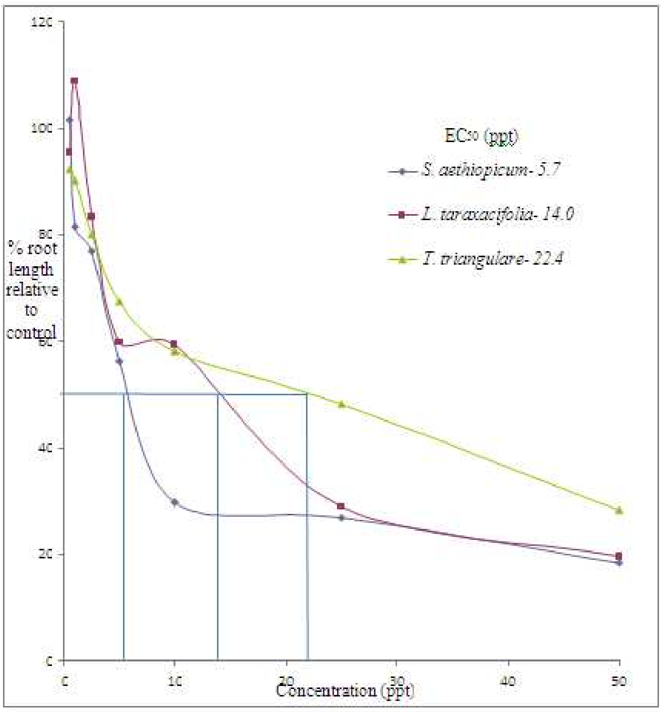
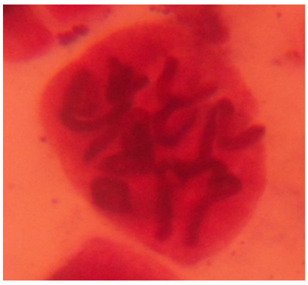
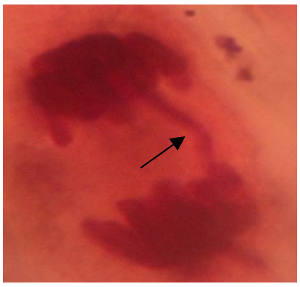
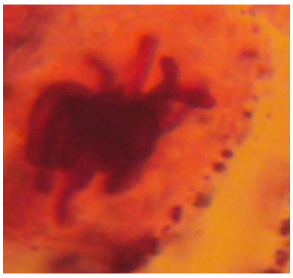


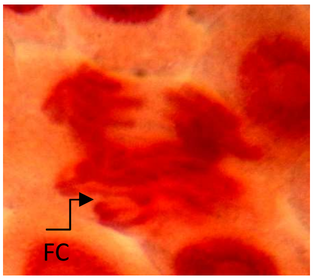
Figure 1: The relationship between percentage root lengths of *A. cepa* root treated separately with *S. aethiopicum*, *L. taraxacifolia* and *T. triangulare* relative to control.





a: c-mitosis b: chromosome bridge





c: sticky chromosome d: fragmented chromosme

Figure 2: Chromosomal aberrations observed in *Allium cepa* root cells treated with vegetable extracts

Table 4: Summary of the cytological effects of aqueous extract of *S. aethiopicum* on *A. cepa* root cells

Concentration (ppt)	No. of dividing cell	Mean of dividing cells X(±S.E)	% of Mitotic index	% of Mitotic inhibition	SC	DS	CB	CL	CM	CF	TAC	% FOA
Control	270	$54.0^{ab}(\pm 3.65)$	5.40	-	-	-	-	-	-	-	-	-
0.5	249	$49.8^{abc}(\pm 9.16)$	4.98	7.78	-	-	-	-	-	-	-	-
1.0	207	$41.4^{bc}(\pm 2.91)$	4.14	23.33	-	-	-	-	1	-	1	0.02
2.5	296	$59.2^{ab}(\pm 5.06)$	5.92	-9.63	-	-	-	-	-	-	-	-
5.0	199	$39.8^{bc}(\pm 3.15)$	3.98	26.30	-	-	-	-	3	-	3	0.06
10.0	194	$38.8^{c}(\pm 6.11)$	3.88	28.15	-	-	-	-	-	-	-	-
25.0	65	$13.0^{d}(\pm(0.89)$	1.30	75.93	-	-	-	-	-	-	-	-
50.0	1	$0.2^{d}(\pm 0.20)$	0.02	99.63	-	-	-	-	-	-	-	-

SC- Sticky chromosome, TAC- Total aberrant cell, Conc.- Concentration, DS- Disturbed Spindle, FOA- Frequency of aberration, CM- C-mitosis, CB- Chromosome bridge, CF- Chromosome fragment, CL- Chromosome lag
Mean value with different alphabets were significant based on Duncan test (P<0.05)

Table 5: Summary of the cytological effects of aqueous extract of *L .taraxacifolia* on *A. cepa* root cells

Concentration (ppt)	No. of dividing cell	Mean of dividing cells X(±S.E)	% of Mitotic index	% of Mitotic inhibition	SC	DS	CB	CL	CM	CF	TAC	% FOA
Control	270	54.0^{ab}(±3.65)	5.40	-	-	-	-	-	-	-	-	-
0.5	173	34.6^{bc}(±2.24)	3.46	35.93	-	-	-	-	1	-	1	0.02
1.0	146	29.2^{c}(±2.20)	2.92	45.93	-	-	-	-	-	-	-	-
2.5	214	42.8^{ab}(±3.22)	4.28	20.74	-	-	1	-	-	-	1	0.02
5.0	221	44.2^{ab}(±4.81)	4.42	18.15	1	-	-	-	1	-	2	0.04
10.0	241	48.2^{a}(±5.11)	4.82	10.74	-	-	-	-	-	-	-	-
25.0	139	27.8^{c}(±(5.23)	2.78	48.52	-	-	1	-	1	1	3	0.06
50.0	156	31.2^{bc}(±5.93)	3.12	42.22	-	-	3	-	-	-	3	0.06

SC- Sticky chromosome, TAC- Total aberrant cell, Conc.- Concentration, DS- Disturbed Spindle, FOA- Frequency of aberration, CM- C-mitosis, CB- Chromosome bridge, CF- Chromosome fragment, CL- Chromosome lag
Mean value with different alphabets were significant based on Duncan test (P<0.05)

Table 6: Summary of the cytological effects of aqueous extract of *T. triangulare* on *A. cepa* root cells

Concentration (ppt)	No. of dividing cell	Mean of dividing cells X(±S.E)	% of Mitotic index	% of Mitotic inhibition	SC	DS	CB	CL	CM	CF	TAC	% FOA
Control	270	54.0^{ab}(±3.65)	5.40	-	-	-	-	-	-	-	-	-
0.5	212	42.4^{a}(±3.44)	4.24	21.48	-	-	-	-	-	-	-	-
1.0	181	36.2^{ab}(±4.04)	3.62	32.96	-	-	-	-	-	-	-	-
2.5	148	29.6^{bc}(±5.08)	2.96	45.19	-	-	-	-	-	-	-	-
5.0	95	19.0^{c}(±4.67)	1.90	64.81	-	-	2	-	-	1	3	0.06
10.0	127	25.4^{bc}(±1.03)	2.54	52.96	-	-	-	-	-	-	-	-
25.0	116	23.3^{c}(±1.99)	2.32	57.04	1	-	1	-	1	-	3	0.06
50.0	121	24.2^{bc}(±5.13)	2.42	55.19	4	-	-	-	-	-	4	0.08

SC- Sticky chromosome, TAC- Total aberrant cell, Conc.- Concentration, DS- Disturbed Spindle, FOA- Frequency of aberration, CM- C-mitosis, CB- Chromosome bridge, CF- Chromosome fragment, CL- Chromosome lag
Mean value with different alphabets were significant based on Duncan test (P<0.05)

Table 3: Summary of the inhibitory effects of the extract of *S. aethiopicum, L. taraxacifolia* and *T. triangulare* on root length of *A. cepa*

	S. aethiopicum				*L. taraxacifolia*				*T. triangulare*			
Conc. (ppt)	Overall no. of roots	Overall mean X(± S.E× 1.95)	% root length relative to control	% root inhibition relative to control	Overall no. of roots	Overall mean X(± S.E)	% root length relative to control	% root inhibition relative to control	Overall no. of roots	Overall mean (X± S.E)	% root length relative to control	% root inhibition relative to control
Control	340	3.20(±0.18)	100	-	340	3.20±0.18	100	-	340	3.20(±0.18)	100	-
0.5	318	3.25(±0.16)	101.56	-1.56	423	3.05±0.16	95.31	4.69	356	2.95(±0.16)	92.19	7.80
1.0	315	2.61(±0.16)	81.56	18.44	426	3.43±0.16	108.75	-8.75	280	2.89(± 0.18)	87.50	9.69
2.5	310	2.46(±0.16)	76.88	23.12	332	2.67±0.18	83.43	16.57	280	2.69(±0.16)	84.06	15.94
5.0	333	1.80(±0.16)	56.25	43.75	324	1.91±0.12	59.69	40.31	327	2.16(±0.14)	67.50	32.50
10.0	260	0.95*(±0.06)	29.69	70.31	301	1.9(±0.1)	59.38	40.62	249	1.86(±0.12)	58.13	35.62
25.0	179	0.86*(±0.06)	26.88	73.12	298	0.93*(±0.08)	29.06	70.94	298	1.54(±0.10)	48.13	51.88
50.0	259	0.59*(±0.04)	18.44	81.56	174	0.63*(±0.04)	19.69	80.31	351	0.91*(±0.04)	28.43	77.56

In conclusion, the data produced herein will provide additional information on the nutritional components and the cytotoxic effects of the aqueous leave extracts of vegetables. More importantly, it will create awareness on the need to be acquainted with information on sources of the vegetable to be consumed.

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