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Archives of Applied Science Research, 2011, 3 (6):383-391
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

Nutritional qualities and cytotoxic evaluation of *Vernonia amygdalina, Amaranthus caudatum* and *Telfairia occidentalis* vegetables widely consumed in South West Nigeria

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ABSTRACT

Nutritional qualities of vegetables, Vernonia amygdalina; Amaranthus caudatum and Telfairia occidentalis, were evaluated along with the cytotoxic effects of their aqueous extracts to ascertain the potential risk that may be associated with the consumption of vegetables from unknown sources. Leaves of the plants were used for proximate analysis, while microscopic and macroscopic tests of the aqueous extracts of each of the vegetables were conducted using 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0 and 50.0 ppt. Physicochemical parameters of the water used in extract preparation and heavy metals of the extracts were also analyzed. Proximate analysis revealed no significant difference in protein, fibre and moisture content of the vegetables. However, ash content in T. occidentalis was significantly different from other vegetables while carbohydrate in V. amygdalina and A. caudatum were similar but different from the value of T. occidentalis. Variations were observed in the values of heavy metals with significant difference in the Fe, Zn and Cu values. Significant inhibitory effect was observed at 5.0, 10.0, 25.0 and 50.0 of all the extracts in addition to 2.5 ppt of V. amygdalina. The EC_{50} values of 8.0, 7.0 and 2.4 ppt were obtained for V. amygdalina, A. caudatum and T. occidentalis respectively which also indicate increasing order of inhibitory effects. Reduction in values of dividing cells and mitotic index of the treated onions were prominent at higher concentrations of the vegetable extracts. Chromosomal aberrations observed include chromosome bridge, laggard chromosome, vagrant chromosome and c- mitosis. The results revealed that the vegetables have high nutritional potential. However, bioaccumulation of toxicants reflects in their potential cytotoxicity on the Allium cepa roots. The data obtained in this study will create awareness for the consumer to know the sources of the vegetables before been purchased for consumption.

Key words: Vegetable, aberration, cytotoxicity, *Allium cepa*, heavy metal, proximate.

INTRODUCTION

Human beings rely on plants for food, drinks, shelter, clothing, equipment and medicines. Large varieties of plants are consumed by man and are used in producing drugs and chemicals like

cosmetics, pesticides, antiseptics and so on [1, 2]. Vegetables are important protective food and highly beneficial for the maintenance of health and prevention of diseases. They contain valuable food ingredients which can be utilized to build up and repair the body. The use of plant extracts as an alternative form of medicinal treatment to orthodox medicine is enjoying great popularity since the late 1990s [3]. In the South West Nigeria, bitter leaf (*Vernonia amygdalina*), amaranths (*Amaranthus caudatum*), and pumpkin leaf (*Telfairia occidentalis*) are among the widely consumed vegetables and are also used in different medicinal applications.

Vernonia amygdalina Del (Bitter leaf plant) of family Asteraceace is a small ever-green shrub found all over Africa. It is widely distributed in Nigeria [4]. Although any part of the plant can be consumed, there seems to be preference for the leaves which are quite bitter in taste due to its chemical contents [5, 6] which is being taken as a sign of potency [7]. It is usually cultivated for its leaf as vegetable, and also for medicinal, traditional and domestic uses.

Amaranthus caudatum L. belongs to the family Amaranthaceae and grows annually as an erect, monoecious herb, of about 100-130 cm tall, much branched and with stem that is obtusely angular. The leaves are simple with alternate arrangement. This group of vegetable is commonly cultivated for their nutritional quality. *A. caudatum* is used in traditional medicine to treat diabetes, internal bleeding, diarrhea and excessive menstruation. The extract from *A. caudatum* has anti-protozoal, anti-inflammatory, anti-oxidant, anti-malaria, and analgesic [8] and anti helminthes [9] properties.

Telfairia occidentalis Hooks F (Fluted pumpkin) of the family Curcubitaceae is a tropical vine grown as a leaf vegetable and for its edible seeds. It is a common tropical green leafy vegetable native to many African countries especially Eastern Nigeria [10]. It has simple, dark green veined leaves and contains nutrients such as proteins, carbohydrates, vitamins, minerals and fibre [11]. The leaf extract has medicinal value and has been used in the management of cholesterolemia, liver problems and impaired immune system [12, 13]. The ability of the plant to combat certain diseases may be due to its antioxidant and antimicrobial properties, and its minerals, vitamins and high protein contents [14].

Despite the nutritional qualities and the medicinal properties of these plants, consumers need to take caution because vegetables generally had been reported to have potential to absorb metals from the soil as well as from deposits on parts exposed to the air from polluted environments [15, 16]. The demand for these vegetables is very high that they are being cultivated using soils from different sources without prior determination of the level of their toxicants. Poultry dropping and inorganic fertilizer are even used in some cases. In most cases, sources of the vegetable purchase from the market are not known to the consumer so also is the level of toxicant accumulation. In this study, the nutritional qualities of *V. amygdalina*, *A. caudatum* and *T. occidentalis* obtained from a commercial market in the South West Nigeria were evaluated. Furthermore, the potential cytotoxic effects of their aqueous extracts were tested on *Allium cepa* root cells.

MATERIALS AND METHODS

Fresh vegetables were obtained from Sabo Market in Ogbomosho and were identified at the Department of Pure and Applied Biology, LAUTECH, Ogbomoso with LHO 201, LHO 202 and LHO 203 respectively assigned to *Amaranthus caudatum*, *Telfairia occidentalis* and *Vernonia amygdalina as* voucher numbers. Extract of each of the vegetables was prepared by boiling 500 g in 1 litre of water for 30 minutes to obtain the stock solution. These were stored in the refrigerator (4^0C) until further analysis.

Physicochemical analysis of water: The well water sample used for extracts preparation and planting of control onion bulbs was subjected to physicochemical analysis. The parameters evaluated include: heavy metals concentration (Pb, Cd, Cr, Ni, Cr, Cu, Zn and Fe), pH, dissolved oxygen, total suspended solids, total dissolved solids, sulphate content, nitrate content, salinity, conductivity, biological oxygen demand and alkalinity [17]

Proximate analysis of the vegetables: Air dried leaves (10g) were used for evaluation of moisture, protein, fat, ash and crude fibre [18] while carbohydrate was calculated by percentage differences.

Heavy metal analysis: Prepared extracts of the vegetables were used for heavy metal analysis. The organic matter was digested with mixture of HNO_3 and HCl **[**19**]** and filtered after cooling. Samples were analyzed using Atomic Absorption Spectrophotometer (Buck 210 AAS, 2005, USA).

***Allium cepa* assay**: The toxic potential of the vegetable extracts was evaluated using *Allium cepa* assay. The onion bulbs used were 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. Eight concentrations (0.25, 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 [20]. The slides were prepared for microscopic evaluation by soften the tissue in I N HCl at 60^0C for 5 minutes, after which it was macerated on a cleaned slide and stained with aceto-orcein for 15 minutes. Cover slip was placed directly on it and the edges were sealed with nail cortex. The dividing cells were scored for different aberrations as previously described [19, 21, 22].

Statistical analysis: Data obtained for root length were analysed using T test while dividing cells were compared using One - way ANOVA and Duncan Multiple Range Test at $P<0.05$ level of significance The statistical package used was SPSS version 15.

RESULTS

The physicochemical properties of the water used in planting of control and extracts preparations compared with the Nigerian Standard Drinking water Quality, NSDWQ, [23] are presented in Table 1. The result showed that all parameters evaluated except Pb were within the allowable limit. However, Cadmium (Cd), Copper (Cu), chromium (Cr) and Nickel (Ni) were not detected. The proximate analysis of leaves of the three vegetables (Figure 1) showed that the ash and carbohydrate content of *V. amygdalina* and *A. caudatum* were similar but significantly different from that of *T. occidentalis* ($P<0.05$). The protein, moisture, fibre and fat contents in the three vegetables showed variations without significant differences in their values ($P>0.05$). Variations were also observed in the mean values of heavy metal composition of the vegetable extracts (Table2). Cu levels in *T. occidentalis and V. amygdalina* were similar but significantly different from the value obtained for *A. caudatum* while Fe in *A. caudatum and T. occidentalis* were similar but different from the value obtained for *V. Amygdalina* ($P<0.05$).

Table 3 shows the growth response of *A. cepa* root to extracts of *V. amygdalina*, *A. caudatum* and *T. occidentalis*. There was dose dependent reduction in the mean root length of *A. cepa*

treated with *V. amygdalina*. T -test analysis showed that the mean root length of *A. cepa* treated with *V. amygdalina* and *T. occidentalis* extracts were significantly different from control at 2.5, 5.0, 10.0, 25.0 and 50.0 ppt while those treated with *A. caudatum* extract were significantly different only at 10.0, 25.0 and 50.0 ppt ($P<0.05$). There were reductions in number of roots at all concentrations of the three extracts compared to control except at 0.05 ppt of A. *caudatum*. The values obtained for EC_{50} were 2.4, 7.0 and 8.0 ppt for *T. occidentalis, A. caudatum* and *V. amygdalina* respectively (Figure 2)

The effects of the vegetable extracts on *A. cepa* root cells are shown in Tables 4, 5 and 6. Dose dependent reduction in number of dividing cells were observed in *V. amygdalina* treated *A. cepa* root cells while inconsistent decrease in the number of dividing cells were observed in *A. caudatum* and *T. occidentalis*. In all the vegetable extracts treated onion roots, least number of dividing cells was observed at the highest concentration of 50 ppt. Significant reduction in dividing cells compared to control was observed at 2.5 of *V. amygdalina* in addition to 5, 10, 25 and 50 ppt of the three extracts. The mitotic indices were lowered in the treated onion root cells at higher concentration of the extracts compared to the control, and were observed to decrease as the concentration increases from 5ppt to the highest concentrations in the three extracts. Although only one aberration was observed in the control, extract treated onion root cells showed various aberrations which include sticky chromosome, Chromosome bridge, vagrant chromosome and c-mitosis similar to our observation in the previous study [19]. Aberrant cells observed were more at the highest concentrations of the extract treated onion root cells except at 50 ppt of *T. occidentalis*.

DISCUSSION

Proximate analysis established that the vegetables evaluated contain high carbohydrate and protein which are very essential in energy production as well as growth and repair of tissues. Ash was also present in substantial amount with significant difference obtained in that of *T. occidentalis*. The value of ash obtained in this study are however higher than some other vegetables such as *Occimum graticimum* (8.00%) and *Hibiscus esculentus* (8.00%) [24]. High ash content is a reflection of the mineral contents preserved in the food materials [25]. The presence of essential metals (Zn, Cr, Fe) in the three vegetables showed that if consumed will supplement other sources of materials for growth and development in the body**.** However, elevated value of Pb which was observed call for concern. Although, physicochemical analysis of the water sample used was within the allowable limit of NSDWQ except for the lead content with 0.04 mg/L. This value of Pb observed in the vegetables could not have been only from the water used, suggesting that the vegetables probably had bioaccumulated some toxicants (Pb inclusive) from the environment through the soil on which the vegetables were planted as well as from deposits on parts exposed to the air from polluted environments [15, 16]. The varying differences in the metal concentrations might be dependent on the absorption capacity of individual vegetables [26].

The results of the macroscopic evaluation showed various degree of inhibition in treated onion bulbs' roots compared to control. The EC_{50} values revealed that *T. occidentalis* with the least value (2.4 ppt) exhibited more inhibitory effects on the root of *A. cepa* than the other extracts. The reduction in number of dividing cells and mitotic index especially at the higher concentrations of the extracts further reflected the inhibitory effects of the extracts. These indicate that the extracts exhibit mitodepressive effect on the cell division of *Allium cepa*. Mitodepressive effect, being the ability to block the synthesis of DNA and nuclear proteins of some plant extracts had earlier been reported [19, 27, 28]. The aberrations observed in this study pointed to the genotoxicity potential of these extracts which probably have effect on the

mechanism of cell division in the *A. cepa* root. Reduction in mitotic activities could be due to inhibition of DNA synthesis [29]. Similar observation on reduction in mitotic index and induction of aberrant cells was reported for *A. cepa* roots treated with the extract of *V. amygdalina* [22]. The effect observed may be attributed to accumulation of toxicants as observed in this study**.** Metals found in the extracts like Pb, Cu and Zn had been implicated to have cytotoxic effects. Pb was reported to be mutagenic [30] while Cu and Zn were reported in several studies to have cytotoxic effects and were implicated in inhibition of growth of *A. cepa* roots [31, 32]. Some of these metals (Cr, Cd, Pb etc) are already known to have individual toxic effect in animal and human systems. Significant genotoxic effects of cadmium chloride in vivo in mice had been reported [33**].** Cadmium [34, 35] and Chromium [36, 37] had been reported to have significant clastogenic and genotoxic effects while Zinc was reported to cause single break in DNA [38] and chromosomal aberrations [39]

Table 1. Physicochemical properties of water sample

Properties	Values	NSDWQ (2007)
pH	6.57 ±0.06	6.5-8.5
DO	8.45 ±0. 78	-
TSS	240 ±13.56	-
TDS	76 ±3.22	500
SO_4^{2-}	4.13 ±0.18	100
NO_3^-	3.586 ± 0.05	50.0
Salinity	0.07± 0.01	-
Conductivity (µS/m)	151 ±14.01	1000.0
BOD	26.1 ±1.34	-
Cd	ND	0.003
Cr	ND	0.05
Pb	0.04 ±0.01	0.01
Ni	ND	0.02
Cu	ND	1.0
Zn	0.258 ± 0.02	3.0
Fe	0.24 ±0.02	0.30

DO: Dissolved oxygen, TSS: Total suspended solid, TDS: Total dissolved solid and BOD: Biological oxygen demand. ND: not detected; Unit of measurement: mg/L

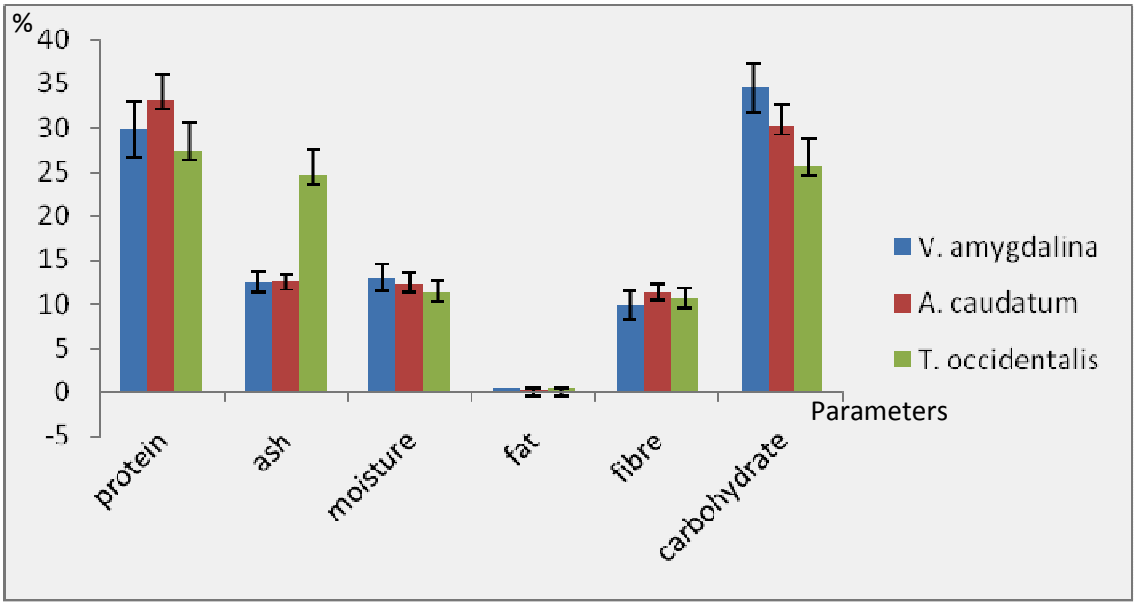

Figure 1. Proximate analyses of the vegetables

The intension of the study is not to scare the consumer but to create awareness on the need to be inquisitive on the sources of the vegetable being bought for consumption. Ingestion of vegetable that has bioaccumulated toxicants especially heavy metals may have undesirable health effects and contribute to the human body burden of these toxicants.

Table 2. Heavy metal analysis of aqueous extracts of *V. amygdalina, A. caudatum* and *T. Occidentalis*

	V. amygdalina	*A. caudatum*	*T. occidentalis*
Fe	1.83^{b}±0.01	3.99^{a}±0.06	4.84^{a} ±0.05
Zn	3.79^{a}±0.12	0.93^{b}±0.07	1.13^{b}± 0.06
Pb	0.05 ±0.01	0.07±0.01	0.09±0.01
Cd	ND	ND	ND
Cr	0.01	ND	0.02
Cu	0.17^{a}±0.02	0.08^{b}±0.01	0.23^{a}±0.01
Ni	ND	ND	ND

ND: not detected; unit: mg/L
Mean value with different alphabets showed significant difference P<0.05

Table 3. Summary of the effects of the extracts of *V. amygdalina, A. caudatum* and *T. occidentalis* on root length of *A. cepa*

Conc. (ppt)	Number of roots	Mean root Length± SE	% root length relative to control	% root inhibition relative to control	Number of roots	Mean root Length± SE	% root length relative to control	% root inhibition relative to control	Number of roots	Mean root Length± SE	% root length relative to control	% root inhibition relative to control
Control	479	2.76±0.08	100	-	479	2.76±0.08	100	-	479	2.76±0.08	100	-
0.25	390	3.06±0.10	110.9	-10.9	445	3.15±0.12	114.1	-14.1	306	2.56±0.14	92.8	7.2
0.5	282	2.48±0.14	89.9	10.1	480	2.50±0.08	90.6	9.4	347	2.94±0.12	106.5	-6.5
1.0	435	2.26±0.08	81.9	18.1	337	2.56±0.08	92.8	7.2	441	2.35±0.10	85.1	14.9
2.5	316	1.84*±0.10	66.7	33.3	357	1.99±0.04	72.1	27.9	144	1.44*±0.10	52.2	47.8
5.0	205	1.63*±0.14	59.1	40.9	341	1.72±0.10	62.3	37.7	210	0.83*±0.06	30.1	69.9
10.0	287	1.17*±0.04	42.4	57.6	182	0.82*±0.12	29.7	70.3	210	0.44*±0.04	15.9	84.1
25.0	157	0.76*±0.08	27.5	72.5	111	0.33*±0.02	12.0	88.0	124	0.30*±0.02	10.9	89.1
50.0	166	0.41*±0.04	14.9	85.1	122	0.36*±0.04	13.0	87.0	71	0.31*±0.08	11.2	88.8
	V. amygdalina				***A. caudatum***				***T. occidentalis***			

**- Significantly different from control using t test (P<0.05); SE- Standard error,*

Table 4. Summary of the cytological effects of *V. amygdalina* on *A. cepa* root cells

Conc. (ppt)	N	Mean X± SE	% of MI	% of MIN	SC	VC	LC	CB	SMLCA	Total AC	% freq. of AC
Cont	293	58.6^{b}±3.42	5.86	-	1	-	-	-	-	1	0.02
0.25	318	63.6^{ab}±3.52	6.36	-8.53	-	1	-	-	-	1	0.02
0.5	362	72.4^{a}±2.75	7.24	-23.55	-	-	1	2	-	3	0.06
1.0	293	58.6^{b}±5.02	5.86	-	-	2	-	1	-	3	0.06
2.5	271	54.2^{bc}±3.54	5.42	7.51	3	-	-	1	-	4	0.08
5.0	226	45.2^{cd}±1.46	4.52	22.87	3	1	-	-	-	4	0.08
10.0	220	44.0^{d}±3.53	4.40	24.91	-	2	-	1	-	3	0.06
25.0	167	33.4^{e}±1.33	3.34	43.00	-	-	-	1	1	2	0.04
50.0	107	21.4^{f}±2.80	2.14	63.48	4	-	-	-	-	4	0.08

Cont: control; MI: mitotic index; MIN: mitotic inhibition; N: number of dividing cells; AC: aberration cells; SC: Sticky chromosome; VC: Vagrant chromosome; CB: Chromosomal bridge, CM: c mitosis, SMLCA: sticky metaphase with looped chromosomal arm
Mean value with different alphabets showed significant difference (P< 0.05)

Table 5. Summary of the cytological effects of *A. caudatum* on *A. cepa* root cell

Conc. (ppt)	N	Mean X± SE	% of MI	% of MIN	SC	CB	LC	CM	Total AC	% freq. of AC
Cont.	293	58.6^{b}±3.42	5.86	-	1	-	-	-	1	0.02
0.25	297	59.4^{b}±4.16	5.94	-1.37	-	-	-	-	-	-
0.5	359	71.8^{a}±4.00	7.18	-2.53	-	-	-	-	-	-
1.0	286	57.2^{b}±6.88	5.72	2.39	1	1	-	-	2	0.04
2.5	285	57.0^{b}±4.14	5.70	2.73	-	-	1	1	2	0.04
5.0	209	41.8^{c}±3.96	4.18	28.67	2	-	-	-	2	0.04
10.0	180	36.0^{cd}±4.09	3.60	38.57	3	-	-	-	3	0.06
25.0	138	27.69^{de}±2.38	2.76	52.90	2	2	-	-	4	0.08
50.0	98	19.6^{e}±2.44)	1.96	66.55	4	-	-	-	4	0.08

Cont: control; MI: mitotic index; MIN: mitotic inhibition; N: number of dividing cells; AC: aberration cells; SC: Sticky chromosome; VC: Vagrant chromosome; CB: Chromosomal bridge, CM: c mitosis, Mean value with different alphabets showed significant difference (P< 0.05)

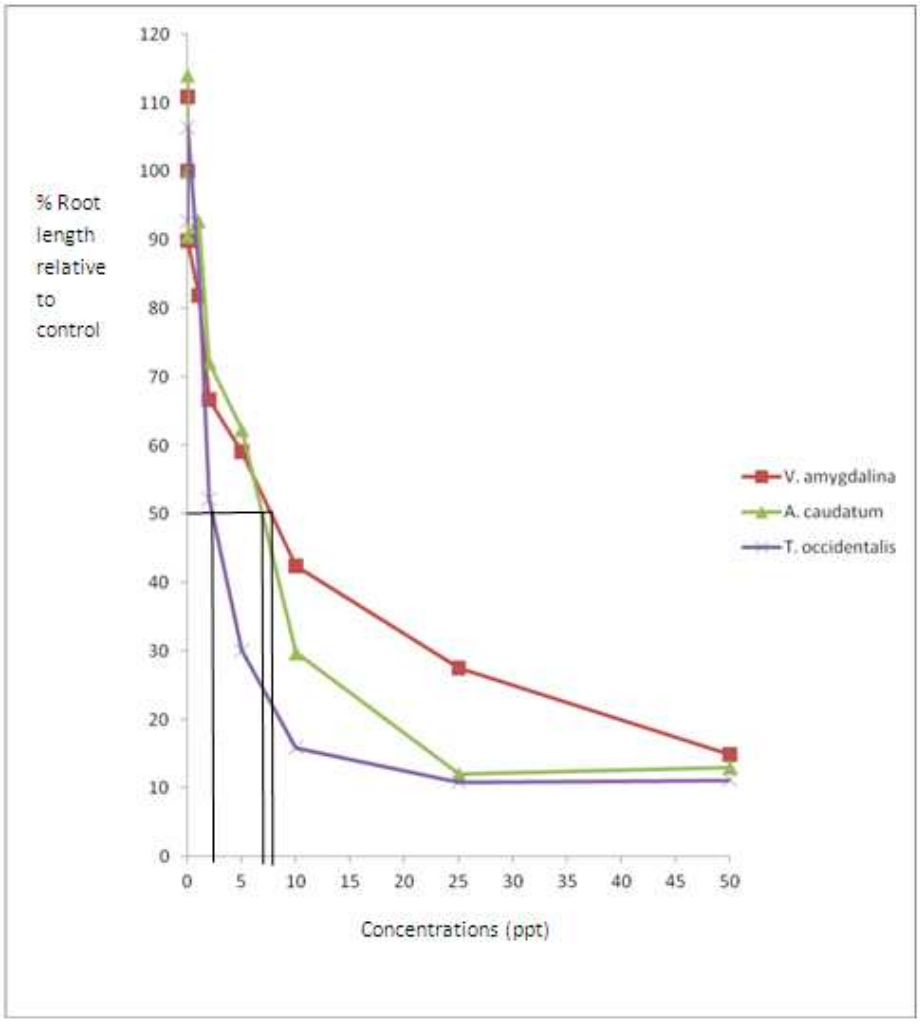

Fig. 2. Percentage root length relationship of *A. cepa* treated with extracts of *Vernonia amygdalina, Amaranthus caudatum* and *Telfairia occidentalis*

Table 6. Summary of the cytological effects of *T. occidentalis* on *A. cepa* root cells

Conc. (ppt)	N	Mean X± SE	% of MI	% of MIN	SC	CB	VC	CM	Total AC	% freq. of AC
Cont.	293	58.6^{b}±3.42	5.86	-	1	-	-	-	1	0.02
0.25	312	62.4^{ab}±2.42	6.24	-6.48	-	-	-	-	-	-
0.5	357	71.4^{a}±3.96	7.14	-21.8	-	-	-	-	-	-
1.0	296	59.2^{b}±2.16	5.92	-1.02	2	-	-	-	2	0.04
2.5	310	62.0^{ab}±3.81	6.20	-5.80	2	1	-	-	3	0.06
5.0	239	47.8^{c}±2.08	4.78	18.43	-	1	1	-	2	0.04
10.0	209	41.8^{c}±3.07	4.18	28.67	2	1	-	1	4	0.08
25.0	137	27.4^{d}±4.90	2.74	53.24	2	-	-	2	4	0.08
50.0	100	20.0^{d}±3.51	2.00	65.87	1	-	-	-	1	0.02

Cont: control; MI: mitotic index; MIN: mitotic inhibition; N: number of dividing cells; AC: aberration cells; SC: Sticky chromosome; VC: Vagrant chromosome; CB: Chromosomal bridge, CM: c mitosis, Mean value with different alphabets showed significant difference (P< 0.05)

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