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EVALUATION OF CYTOGENOTOXIC AND NUTRIENT COMPOSITION OF THREE COMMONLY CONSUMED VEGETABLES IN SOUTH- WESTERN NIGERIA

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ABSTRACT

Consumption of leafy vegetables provides health benefits but sometimes may turn out to be the cause of certain health problems. Thus, adequate toxicological screening is needed to ensure safety of their consumption. Aqueous extracts of *Corchorus olitorius* L., *Celosia argentea* L., and *Ocimum gratissimum* L. were evaluated for cyto-genotoxic effects on *Allium cepa* L. root cells using 0.5, 1.0, 2.5, 5.0, 10.0, 25.0 and 50.0 part per thousand (ppt), each of the vegetable extracts. The levels of macronutrients (moisture, ash, protein, fibre, fat and carbohydrate) in the vegetables and some minerals (cadmium, copper, iron, lead, magnesium, nickel and zinc) of their aqueous extracts were determined. The carbohydrate values were in the order *O. gratissimum* > *C. olitorius* > *C. argentea* with 48.17% as the highest. The values obtained for ash, protein, fat, Mg and Zn contents were in the order *C. argentea* > *C. olitorius* > *O. gratissimum* , with the highest values of 19.98%, 30.79%,, 0.22% , *226.4 mg/L and 2.57mg/L,* respectively obtained for *C. argentea.* The concentration of Cu and Pb were in the order of *O.*gratissium> *C. argentea> C. olitorius with 0.67* and 0.21mg/L obtained for *O.*gratissium *(P<0.05).* The aqueous extracts of the three vegetables inhibited root growth and cell division in the *A. cepa* root tips, with the highest inhibitory effects observed in *C. argentea* at 50.0 ppt. Induced chromosomal aberrations were significant only at 1.0 and 25 ppt of *O. gratissimum*, whereas there were no significant differences in aberrant cells *in C. olitorius* and *C. argentea* compared to the control. Chromosomal aberrations observed in the treated *A. cepa* roots include c-mitosis, Chromosome bridge and sticky chromosome. The results showed that the three vegetables have nutritive qualities but with root growth and mitotic inhibitory activities, which were severe in *C. argentea*. Although the result indicates that chromosomal aberrations might be induced at higher concentrations, the antimitotic potential of the extract of *C. argentea* may favour its uses in the development of drugs to prevent the uncontrolled proliferation of cancer cells of which investigation is required.

Key words: Cytogenotoxicity, minerals, proximate, aberration, *Allium*

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INTRODUCTION

Human diets in most developing countries have green leafy vegetables as one of the indispensable sources of carbohydrate, protein, vitamins and minerals, as well as roughages which promote digestion and prevent constipation [1, 2]. In Nigeria, most of the commonly eaten vegetables are the succulent leaves of herbaceous plants which are consumed as supplements, side dishes, prepared in soup as condiments or eaten with other major staple foods. Different parts, such as roots, stems, leaves, fruits or seeds of vegetables may be edible, and each of them contributes to diet in its own way [3]. Vegetable fats and oils have been reported to lower blood lipids, thereby reducing the occurrences of diseases associated with the damage of the coronary artery [4]. High fruit and vegetable diets have also been reported to have an ameliorating effect on the blood pressure of hypertensive patients [5], lowered the systolic and diastolic blood pressure, blood viscosity and plasma fibrinogen [6].

Corchorus olitorius (L.), *Celosia argentea* var. *cristata* (L.), and *Ocimum gratissimum* (L.) are among the commonly consumed vegetables in the South-West zone of Nigeria. *C. olitorius* is usually recommended for pregnant women and nursing mothers due to its richness in iron [7]. *C. argentea* is used as vegetable and ornamental plant and also, its dried leaves, inflorescences and seeds are used in traditional Chinese medicine [8]. Celogentins and moroidin, the bicyclic peptides isolated from the seeds of *C. argentea*, showed potent inhibition of tubulin polymerization, suggesting antimitotic activity of these compounds [9.10]. The genus *Ocimum* belongs to the economically important group of herbaceous plants widely distributed in tropical Africa and being grown in different parts of Nigeria. In African traditional medicine, the aqueous leaves decoction of *O. gratissimum* is used to treat gastrointestinal disorders and helminthiasis [11] and also has antioxidant properties [12]. Eugenol, an essential oil of *O. gratissimum*, demonstrated leishmanicidal activity and was not cytotoxic against mammalian cells [13]. However, in another report, *Ocimum* oil was reported to be capable of invoking an inflammatory response that transits from acute to chronic toxicity if continuously administered [14].

Although vegetables contain essential nutrients for human health benefits, some are toxic, or contain constituents that compromise digestion and absorption of vital nutrients [15]. The toxic constituents of vegetables may act on the chromosomes, thereby causing serious genetic problems such as cancers in the affected individuals or inheritance of abnormal genes in their offspring [16]. Proximate and mineral composition of the selected vegetables used in the present study have been reported in different locations around Ogbomoso, Nigeria [2, 17, 18] but none of the investigations included *in vivo* cyto-genotoxic evaluations. It is on this basis that the further screening of these common edible vegetables; *C. olitorius, C. argentea* and *O. gratissimum* for bioaccumulation of toxic minerals coupled with the genotoxicity of their aqueous extracts were conducted. This is with a view to give an insight into possible hazards associated with their consumption either as part of human diet or for medicinal purposes.

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MATERIALS AND METHODS

Collection of samples

Three vegetables namely: *Corchorous Olitorious* (L.), *Ocimum gratissimum* (L.) and *Celosia argentea* var. *cristata* (L.) were freshly obtained from 'Wazo' market in Ogbomoso, Oyo state, Nigeria and were identified at the herbarium, LAUTECH, Ogbomoso.

Physicochemical analysis of water used: The water sample used for extracts preparation and planting of control onion bulbs was subjected to physicochemical analysis. The parameters evaluated included: pH, dissolved oxygen, total suspended solids, total dissolved solids, sulphate content, nitrate content, salinity, conductivity, biological oxygen demand and alkalinity [19]. The concentrations of the selected metals (Cd^{2+}, $Cr^{+2}Cu^{2+}$, Fe^{2+}, Pb^{2+}, Mg^{2+}, Ni^{2+} and Zn^{2+}) were also analyzed.

Preparation of extract

Five hundred grams (500g) each of the vegetables were weighed and boiled separately in 1L of borehole water. The aqueous extracts obtained were allowed to cool, after which the extracts were filtered and kept at 4 °C for experimental use.

Proximate analysis of the three vegetables

Moisture, ash, protein, fibre, fat and carbohydrate contents of the studied vegetables were determined using the standard method [20]. All the proximate values were calculated and recorded [2, 21].

Metal contents analysis of the three vegetables

The presence and concentrations of Cd^{2+}, $Cr^{+2}Cu^{2+}$, Fe^{2+}, Pb^{2+}, Mg^{2+}, Ni^{2+} and Zn^{2+} in the three vegetables were determined by wet digestion and analyzed using Atomic Absorption Spectrometer - AAS (PerkinElmer A Analyst 100). Standards for the analyzed metals were prepared from a known concentration of 1000 µg/ml [21]. The samples analyses were carried out following the appropriate quality assurance procedures and precautions in order to ensure reliability of the data [2].

Allium cepa assay

Onions bulbs (*Allium cepa*) were purchased from 'Wazo' market in Ogbomoso, Nigeria and sun dried for three weeks before being used for the experiment. The dried outer scales and roots in the onions were removed carefully, leaving the primordial root ring intact. The stock extract was diluted using the same water that was used for the control group into seven concentrations; 0.5, 1.0, 2.5, 5.0, 10.0, 25.0 and 50.0 ppt. Ten onion bulbs were planted per concentration of each of the extracts in a dark cupboard at room temperature. After 24 h, fresh extract solution was prepared and the previously suspended onions were transferred thereafter for the root growth to continue [22, 23].

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Microscopic evaluation
After 48 h of root growth, root tips from five out of ten onions per concentration were cut, fixed separately in ethanol-ethanoic acid (ratio 3:1) fixative and stored at 4 °C for slide preparation. The fixed root tips were first hydrolyzed in 1N HCl and further treated as previously described [22]. Two root tips were teased on a clean glass slide using a pair of needles. One - two drops of aceto-orcein stain was added to the homogenized root tips and left for 10 minutes. Thereafter, excess stain was removed with a filter paper and a cover slip was carefully placed on the slide. The slide was wrapped with rolls of tissue paper and tapped with an index finger to remove excess stain, after which the edges of the cover slip were then sealed with nail polish. Five slides were prepared and scored per concentration. A total of 5000 cells per concentration were examined for dividing cells and chromosomal aberrations using oil immersion objective lens. Mitotic index and frequency of chromosomal aberrations were calculated as reported [23].

Macroscopic evaluation
Root lengths from the remaining five onions per concentration were measured after 72 h. The average root lengths obtained for the treated and control groups were calculated and used for plotting a graph of percentage root length against concentration (22). Therefore, the level of toxicity of aqueous extracts on the root growth in *A. cepa* was determined from the graph.

Statistical method
The data obtained from proximate and metal analyses, as well as the root lengths, mitotic indices and frequency of chromosomal aberrations of the treated groups and the control were compared using one way analysis of variance (ANOVA) and Duncan's multiple range test. The difference between the control and treated groups was considered significant at $p \leq 0.05$. All statistical analyses were performed with SPSS software, version 15.0.

RESULTS

The proximate analyses of the studied vegetables (Table 1) showed that carbohydrate was the most abundant with the value obtained for *C. argentea* (31.41%) significantly lower compared to other two vegetables. The values of protein (30.79%), ash (19.98), and fat (0.22%) obtained for *C. argentea* were significantly different from those obtained for *O. gratissimum* and *C. olitorius* vegetables, while the highest moisture content (8.85%) was found in *O. gratissimum.*

The concentrations of Magnesium and Zinc in *C. argentea* were significantly different from the other two vegetables. There were no significant differences in the values of Iron in *C. olitorius* and *C. argentea* both of which were 20.86 mg/l, and values of lead obtained for *O. gratissimum* (0.21mg/l) and *C. argentea* (0.20 mg/l). Cadmium and Nickel were not detected in the three vegetables (Table 2). The physicochemical analysis of the bore-hole water used as control and diluent for *A. cepa* assay is shown

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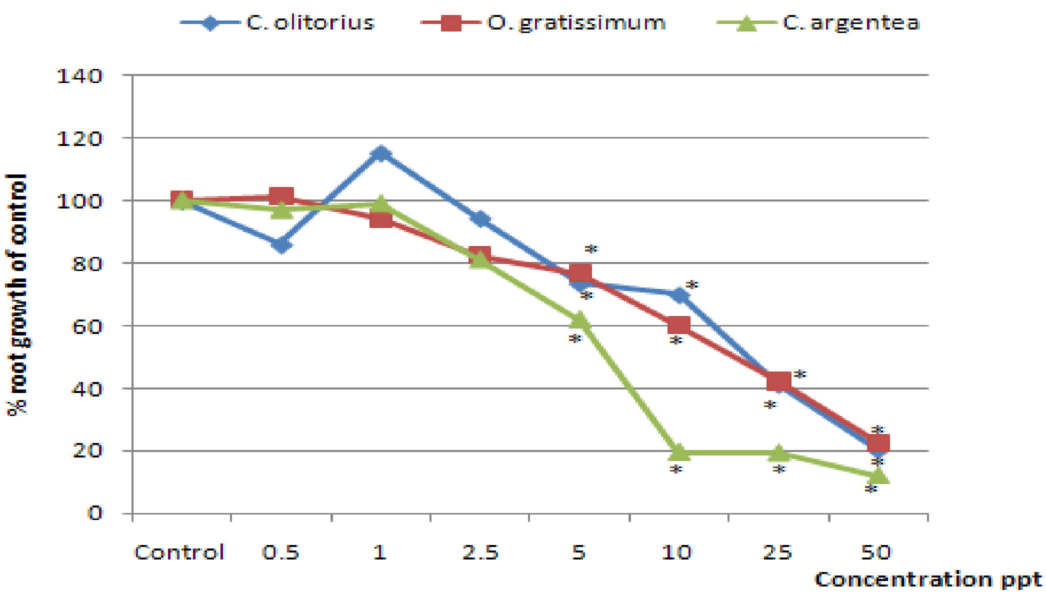
in table 3. The values obtained for Zn (0.248 mg/l) and Fe (0.26mg/l) in the bore-hole water were lower compared to values obtained for the vegetables extracts while Cd, Cr, Pb, Ni and Cu were not detected.

The toxicity of aqueous extracts of *C. olitorius*, *O. gratissimum* and *C. argentea* to the root growth of *A. cepa* is represented in Figure 1. The values obtained for EC_{50} were 6.3, 19.0 and 22.4 ppt for *C. argentea*, *O. gratissimum* and *C. olitorius*, respectively. The percentage root growth inhibitions caused by the aqueous extracts of the three vegetables at 5.0, 10, 25 and 50 ppt was significantly different ($p \leq 0.05$) from the control.



* significant difference ($P \leq 0.05$)

Figure 1: Effects of aqueous extracts of *C. olitorius, O. gratissimum* and *C. argentea* on root growth of *Allium cepa*

Figure 2 shows the activity of the aqueous extracts of *C. olitorius*, *O. gratissimum* and *C. argentea* in suppressing mitosis in the root tips of *A. cepa*. Significant reduction in the number of dividing cells was observed at all concentrations of *C. argentea* whereas, the significant inhibition of mitosis occurred at 25 and 50 ppt of *C. olitorius* and 2.5, 10, and 50 ppt of *O. gratissimum.* Inhibition of mitosis in *A. cepa* by the aqueous extracts of *C. olitorius* and *O. gratissimum* was not concentration dependent, unlike the mitotic inhibitory effect of aqueous extract of *C. argentea,* which was dose dependent from 2.5 ppt to 50 ppt.

The aqueous extracts of *C. olitorius*, *O. gratissimum* and *C. argentea* induced the following chromosomal aberrations; c-mitosis, chromosome bridge and sticky chromosome, in the *A. cepa* root tip cells (Table 3). The frequencies of the

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aberrations; 0.20% and 0.14% recorded at 1.0 ppt and 25.0 ppt, respectively for the aqueous extract of *O. gratissimum*, were significantly different from the control, while no chromosomal aberration was induced at 50.0 ppt of the extract. Similarly, no chromosomal aberration was induced at the first four concentrations of *C. argentea*, while those aberrations induced at the last three concentrations of *C. argentea* were not significantly different from the control, which had no aberration. The induced chromosomal aberrations by these vegetables were not dose-dependent.

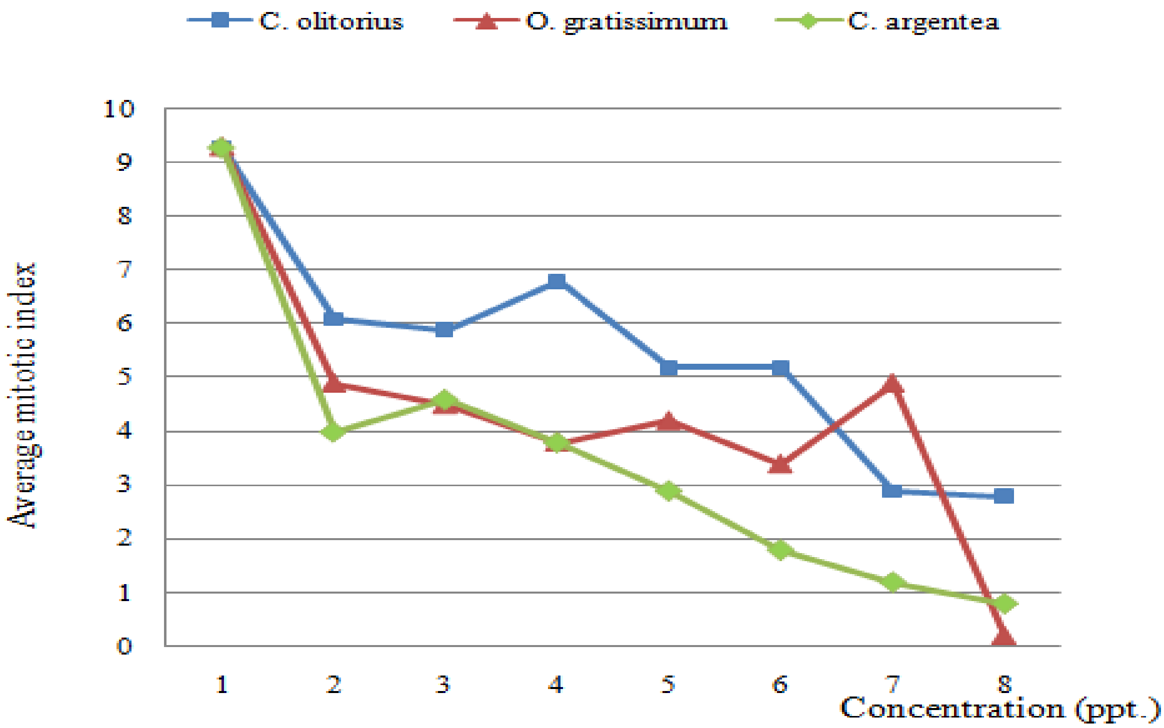

Figure 2: Average number of dividing cells induced by aqueous extracts of *C. olitorius*, *O. gratissimum* and *C. argentea* in the root tips of *Allium cepa*

DISCUSSION

Green leafy vegetables provide humans with adequate amounts of many vitamins and minerals and thus occupy an important place among the food crops. They are valuable in maintaining alkaline reserve of the body because of their nutrient and mineral contents [2]. Among the nutrient and mineral contents analyzed in this study, the percentage of carbohydrate and protein in the three vegetables ranged from 31.42 - 49.39% and 22.24 - 30.79%, respectively. The results are higher than the carbohydrate and protein contents of *C. olitorius* and *C. argentea* reported [2]. The protein contents of the three vegetables are within the range of 20.48 - 41.66% reported as the protein level of green leafy vegetables [25]. Plant food that provides more than 12% of its calorific value from protein is considered good source of protein

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[26]. Therefore, these results suggest that *C. olitorius*, *O. gratissimum* and *C. argentea* are good source of protein and carbohydrate for humans, especially in developing countries where most people depend on starch-based food as the main staple food for the supply of both energy and protein [2]. The moisture (7.16 - 8.85%) and ash (10.79 - 19.98%) contents of these vegetables are lower than the values reported for *C. olitorius*, *C. argentea* [2, 18] and *O. gratissimum* [27]. The difference in the sampling time and location of vegetables may be responsible for the disparity in the results of moisture contents. The fibre contents of *C. olitorius* (7.40%) and *C. argentea* (10.22%) are almost similar to 6.70% and 11.70% previously obtained for these two vegetables, respectively [2]. Dietary fibre has been reported to lower the risk of coronary heart disease, hypertension, constipation, diabetes, and colon and breast cancer [28, 29]. Different fat content from the one obtained in this study was earlier reported for *C olitorius* [2]. *O. gratissimum* had the least fat content of 0.09%. Similarly, the lowest values of fat content among the analyzed nutrients in the vegetables studied had been observed in *Amaranthus cruentus*, *Celosia argentea* and *Corchorus olitorius* leaves [2]. The results of proximate analysis on these three vegetables showed that they could be good for health by providing most of the essential nutrients for normal body functions when consumed in appropriate combinations.

The highest magnesium content, followed by iron, suggests health benefits of the vegetables to their consumers in terms of cellular metabolism and protein synthesis. However, the levels of Pb^{2+} (0.14 - 0.21 mg/L) which are lesser than the suggested concentration of 2 – 6 mg/l in the plant species [30], as well as the lack of Cd^{2+} and Ni^{2+}, corroborates the use of these vegetables as food supplement. The observed values for the metals evaluated could only have been from bioaccumulation by the vegetable since the physicochemical analysis of the water especial in metal content were lower compared to those of the vegetables and were within the permissible levels of Nigerian Standard for Drinking water Quality (24).

The activity of aqueous extracts of the vegetables on the root growth of *A. cepa* shows inhibitory effects as indicated by the significant ($p \leq 0.05$) root growth inhibition observed at 5.0, 10.0, 25.0 and 50.0 ppt of the three vegetables. The aqueous extract of *C. agentea* demonstrated highest root growth inhibitory effect, as indicated by its lowest EC_{50} value. The root growth inhibitory activities of the aqueous extracts in this study might be due to the presence of some heavy metals. Heavy metals such as Zinc, Copper and Lead at different concentrations have been implicated in inhibition of root growth in *Allium cepa* [31, 32]. Similar root growth inhibitory effects of aqueous extracts of *Vernonia amygdalina*, *Amaranthus caudatum* and *Telfairia occidentalis* in *A. cepa* have been reported [22].

The significant ($p \leq 0.05$) reduction in values of mitotic index obtained at various concentrations of the vegetables compared to the control suggests mitodepression. The concentration-dependent (1.0 – 50.0 ppt) inhibition of mitosis in the root tips of *A. cepa* by the aqueous extract of *C. argentea* is an indication of cytotoxicity, whereas the antimitotic activity of the aqueous extracts of *C. olitorius* and *O. gratissimum* was

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less, and could be regarded as cytostatic because of their non-dose related suppression of mitosis. Celogentins and moroidin are bicyclic peptides isolated from the seeds of *C. argentea*, and have been implicated in the inhibition of microtubule formation, hence, arrest of mitosis in eukaryotic cells [9, 10]. Therefore, the presence of these phytochemicals in the aqueous extract of *C. argentea* may be responsible for the inhibition of mitosis in *A. cepa* cells as observed in this study. This corroborates the higher toxicity to root growth of *A. cepa*, by the extract of *C. argentea* compared to the aqueous extracts of the other two vegetables. It has been observed that whenever there is root growth inhibition in the *A. cepa*, there is always reduction in the number of dividing cells [23, 32, 33].

The induced chromosomal aberrations by the aqueous extracts of these vegetables were not concentration dependent except at 10.0, 25.0 and 50.0 ppt of *C. argentea*. Although aqueous extracts of *O. gratissimum* induced chromosomal aberrations at 1.0 and 25.0 ppt concentrations which were significantly different from control, yet, its mutagenic effects as well as that of *C. olitorius* could be considered weak on the basis of their induction of chromosomal aberrations in a non-dose related manner. However, the potency of the aqueous extract of *C. argentea* in inducing chromosomal aberrations in *A. cepa* cells may be significant and more worrisome at higher concentrations than those used in this study. The ability of celogentins and moroidin in inhibiting the polymerization of tubulin has been reported in cells [9, 10]. This may disallow the assemblage of sipindle fibres, thereby causing chromosomal aberrations. Occurrence of these aberrations in *A. cepa* indicate that similar observations might be possible in higher eukaryotic organisms, human inclusive, if exposed to substantial concentration since the basic structure of the DNA are the same in all organisms.

CONCLUSIONS

This study revealed that *C. olitorius*, *O. gratissimum* and *C. argentea* are nutritious and good for human consumption, as they contain various classes of food, without possessing harmful levels of heavy metals. However, it is worth mentioning that the phytochemicals in these vegetables could significantly suppress cell division in eukaryotic cells, and may induce chromosomal aberrations at higher concentrations. Nonetheless, the antimitotic potential of the extract of *C. argentea* may favour its uses in the development of drugs to prevent the uncontrolled proliferation of cancer cells of which further investigation is required.

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Table 1: Nutritional components (%) of *Corchorus olitorius, Ocimum gratissimum* and *Celosia argentea* used for the study

Vegetable	Moisture	Ash	Protein	Fibre	Fat	Carbohydrate
C. olitorius	7.16±0.08	11.97±0.52^{b}	25.18±2.12^{b}	7.40±0.02	0.12±0.03^{b}	48.17±2.23^{a}
O.gratissimum	8.85±0.12	10.79±0.82^{b}	22.24±1.75^{b}	8.64±0.61	0.09±0.01^{b}	49.39±1.07^{a}
C. argentea	7.38±0.05	19.98±1.04^{a}	30.79±2.72^{a}	10.22±0.42	0.22±0.06^{a}	31.41±1.92^{b}

Data were presented using mean ± Standard error of mean

Mean value with different alphabet showed significant difference ($p<0.05$)

Table 2: Analyzed metals in *Corchorous olitorius*, *Ocimum gratissimum* and *Celosia argentea* used for the study

Vegetable	Cd^{2+}	Cu^{2+}	Fe^{2+}	Pb^{2+}	Mg^{2+}	Ni^{2+}	Zn^{2+}
C. olitorius	ND	0.42±0.02	20.86±1.73^{a}	0.14±0.00^{b}	215.33±15.76^{a}	ND	1.83±0.12^{b}
O.gratissium	ND	0.67±0.00	10.76±0.98^{b}	0.21±0.04^{a}	184.21±8.86^{b}	ND	1.75±0.02^{b}
C. argentea	ND	0.57±0.01	20.86±1.20^{a}	0.20±0.01^{a}	226.40±12.56^{a}	ND	2.57±0.17^{a}

ND: Non detectable; Unit of measurement: mg/L

Mean value with different alphabet showed significant difference ($p<0.05$)

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Table3: Physicochemical characteristics of bore-hole water used for the preparation of vegetable extracts and *A. cepa* root growth solutions

Parameters	pH	DO	BOD	Alkalinity	TDS	TSS	TS	SO_4^{2-}	NO_3^-	Cd	Cr	Pb	Ni	Cu	Zn	Fe
Bore hole water	6.97	6.8	2.67	67.58	108	74	182	4.13	3.86	ND	ND	ND	ND	ND	0.248	0.26
	±0.1	±0.4	±0.04	±1.02	±6.9	±8.08	±9.2	±0.15	±0.04						±0.01	±0.02
NSDWQ (2007)[24]	6.5-8.5	-	-	-	500	-	-	100	50	0.003	0.05	0.01	0.02	1.0	3.0	0.3

Unit: mg/l except pH.
DO: Dissolved oxygen; BOD: Biological Oxygen Demand; TDS: Total Dissolved Solid; TSS: Total Suspended Solid, TS: Total Solid, Cd: Cadmium, Cr: Chromium, Pb: Lead, Ni: Nickel, Zn: Zinc, Fe: Iron

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Table 4: Types and frequencies of chromosomal aberrations induced by aqueous extracts of the three vegetables used for the study

Concentration	C-mitosis	Chromosome bridge	Sticky chromosome	Total aberrations	Frequency of aberrant cells
Control	0	0	0	0	0.00
0.5 ppt					
C. olitorius	0	1	0	1	0.02
O. gratissimum	5	0	0	5	0.10
C. argentea	0	0	0	0	0.00
1.0 ppt					
C. olitorius	0	0	0	0	0.00
gratissimum	6	0	4	10	0.20*
C. argentea	0	0	0	0	0.00
2.5 ppt					
C. olitorius	0	0	0	0	0.00
gratissimum	4	0	0	4	0.08
C. argentea	0	0	0	0	0.00
5.0 ppt					
C. olitorius	0	0	0	0	0.00
gratissimum	4	0	0	4	0.08
C. argentea	0	0	0	0	0.00
10.0 ppt					
C. olitorius	1	1	0	2	0.04
gratissimum	0	0	0	0	0.00
C. argentea	1	0	0	1	0.02
25.0 ppt					
C. olitorius	0	0	0	0	0.00
gratissimum	5	2	0	7	0.14*
C. argentea	1	0	0	1	0.02
50.0 ppt					
C. olitorius	1	2	0	3	0.06
gratissimum	0	0	0	0	0.00
C. argentea	0	0	2	2	0.04

* Values significantly different from control ($p \leq 0.05$)

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