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Assessment of the Mutagenic and Cytotoxic Ability of Azanza Garckeana (F.Hoffm), Using Allium Cepa (L) Assay

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Abstract:
The study on the assessment of the mutagenic and cytotoxic ability of Azanza garckeana leaf and fruit extracts on the root tip of Allium cepa was evaluated; aqueous leaf and fruit extract of A. garckeana were collected and dried, and pounded into powder. For the root growth inhibition and cytological analysis, four concentrations of each extract, viz: 5g,10g, 15g, and 20g, including positive and negative control, were considered, and the result was determined for each extract using a graph to indicate the maximum and minimum EC50 (Effective concentration). The EC ranged from 10 g/ml with a root length of 3 cm for A. garckeana leaf extracts, 10g/ml with a root length of 2.5 cm for A. garckeana fruits extracts, A range of chromosomal aberrations such as polyploidy, dissolution, vagrant metaphase, binucleate, laggard, nuclear lesion, fragmentation, chromosome gap, anaphase bridge, micronuclei, unequal separation, were observed in all the plant's extracts. Statistically, the chromosomal aberrations observed in the root tip cells of A. cepa treated with the leaf extracts of A. garckeana shows that 10g is statistically significantly different from 5g (P = 0.026) and negative control, 15g is significantly different from 5g (P = 0.000) and 10g (P = 0.026). However, as the concentration increased to 20g, there was a significant difference in the percentage of aberrations from 5g (P =0.0000) to 10g (P = 0.005), but not significantly different from 15g (P =0.0553). Whereas fruit extract shows that 10g is higher than 5g but has no statistical significance (P =0.101). 15g is significantly different from 5g (P =0.101) but not significantly different from 10g (P =0.055), 20g (P =0.331), and positive control (P =0.845). As the concentrations of the plant extracts increased to 10%, 15%, and 20%, there was a progressive increase in the percentage of aberrant cells and a decrease in the number of dividing cells, suggesting that low concentrations of A. garckeana extracts should be used in traditional medicine production.

Keywords: *Azanza garckeana, Allium cepa, chromosome aberrations, mutagenic and cytotoxic*

1. Introduction

The use of plants for medicine is probably as old as humankind itself. Plant extracts and other alternative medical therapies have become increasingly popular in recent years worldwide. Generations of people have utilized medicinal plants to treat illnesses, and recently, scientific evidence has been collected that shows many herbs and related key medicinal characteristics are effective in preventing diseases or alleviating their symptoms. (Tougnolini *et al*., 2006). Interest in medicinal plants amongst local communities has increased globally because of their value in the treatment of diseases (Arise *et al*., 2009).

Medicinal plants have played a pivotal role in the health care of many cultures, both ancient and modern (Newman & Cragg, 2007). The WHO defines a medicinal plant as any plant with components in one or more of its organs that can be utilized therapeutically or serve as building blocks for the manufacture of chemo-pharmaceuticals. Secondary metabolites from medicinal plants have been identified and tested for in vitro and in vivo efficacy against a variety of infections. (Madhava *et al*., 2008). United Nations Organization (1990) states that 80% of African use traditional medicine for their primary health care.

The *Allium cepa* assay is also a short-term in vivo cytotoxicity test that tests cytotoxicity using chromosomes and therefore detects chromosome structural and numerical alterations (Tedesco & Laughinghouse, 2012; Bonciu *et al*., 2018). The assay is relatively inexpensive, fast, and gives reliable results, and chemicals that cause chromosomal aberration (CA) in plant cells also produce CA in cultured animal cells that are frequently identical (Ma *et al*., 1994). *Allium cepa* is the common onion introduced by Levan (1938) as an effective test system applicable for toxicity assessment. This plant assay has several intriguing qualities, including sensitivity to complicated mixes, unambiguous chromosomes (2n=16), cost-

effectiveness, and accessibility. The majority of plants produce poisonous chemicals as a defense mechanism against insects and herbivores. Additionally, harmful compounds may have an impact on organisms that consume them, such as humans. Consequently, it makes sense that some medicinal herbs might inhibit the effects of mutagens while other ones might have harmful or mutagenic effects. (Vicentini *et al.*, 2001).

Azanza garckeana (F. Hoffm.)Exell and Hcllc are among the popular multipurpose fruit trees in Africa, characterized by edible fruits with different plant products sold to local markets generating substantial incomes for households (Ahmed *et al*., 2016). Local populations have relied on wild edible fruits for generations in developing nations and marginalized areas as food, medicine, and nutritional supplements. Alfred Maroyi (2017). The medicinal properties of *A*. *garckeana* include their antioxidant effects which play a vital role in preventing chronic ailments such as heart disease, cancer, diabetes, hypertension, stroke, and Alzheimer's disease by combating oxidative stress, Augustino *et al*., (2011). English names for Azanza garckeana include tree hibiscus, Azanza, and snot apple. Its name in Nigeria is gorontula (Orwa et al., 2009; Ochokwu et al., 2014). One of the indigenous fruit tree species found in Nigeria, it is cultivated in a range of soils and can be found close to termite mounds and barren places (Ochokwu et al., 2014). In Gombe State's Kaltungo local government area and Adamawa State's Michika local government area, Nigeria is semi-domesticated. (Ochokwu *et al*., 2015). This research is aimed at determining the minimum and maximum effective concentration (EC50) of *Azanza garckeana,* leave, and fruit extracts on the root tips of *Allium cepa*, and the mutagenic potential of *Azanza garckeana* plant extracts.

2. Material and Method

2.1. Sampling Site for the Medicinal Plants (Collection and Authentication of Plant Samples)

Leaves of *Azanza garckeana* were collected from Tula town of Kaltunago Local Government area of Gombe state. The collected plant specimen was moved down to the department of plant science at Modibbo Adama University for identification using the preserved specimen voucher. The harvested plant material was given a code. The leaves of the collected medicinal plants were washed, dried, and stored under shade in a greenhouse at the plant Science Department Modibbo Adama University, Yola Adamawa State.

2.2. Preparation of Aqueous and Plant Extracts

The leaves and fruits of *A.garckeana* were shade dry and pounded into powder using a pestle and motor. The water extracts of three medicinal plants were prepared by soaking 100 grams of the dried and pounded plant material in 1 liter of water for 72 hours and were filtered using Whitman No 1 paper. The filtered will be kept in the refrigerator until when needed for use.

2.3. Allium Cepa Assay (Determination of Cytotoxicity and Mutagenic Potential)

Onion bulbs (*Allium cepa*, L.) were obtained commercially at Jimeta's main market. They were sun-dried for 4 days, and then the dried outer scales were carefully removed, and the root was scraped, leaving the ring of the primordial root intact to promote the emergence of new roots. These were used for the bioassay according to standard procedures (Akinboro & Bakare, 2009). For the root growth inhibition, four concentrations of each extract, viz: 5g, 10g, 15g, and 20g, were considered. Six onion bulbs were utilized for the concentration of the plant extract, with the positive control group treated with different concentrations of glyphosate and the negative control group treated with tap water. The base of each of the bulbs was suspended on the extract inside 150 mL beakers in the dark for 96 hours. The test extracts were changed daily, and the root length was measured at the end of the exposure period; the length of the roots of five onion bulbs with the best growth at each concentration was measured (in cm) with a ruler. The average length for each concentration and the control was obtained, and the percentage root growth inhibition between the negative and positive control and the EC50 (the effective concentration where root growth amounts to 50 % of the controls) for the extract was also determined. The effect of each sample on the morphology of growing root cells was also examined according to the protocol described by Sibhghatulla and his team (2012).

2.3.1. Laboratory Germination

Seeds were germinated in perforated plastic containers with very fine loam soil to obtain root tips for cytological analysis, break dormancy in the seeds and allow for quick germination. Seeds were pre-soaked in 1% potassium hydroxide for 12 hours watering was done on a daily basis until germination.

2.3.2. Pretreatment and Fixation

When the germinating roots reached 2.5cm long, they were excised using the pointed end of a pair of forceps. This excised was done between 7.30 – 8.30 am when cells of the growing roots are known to be in active division (Oyewole, 1984). The excised root tip was immediately dropped into a solution of 0.05% colchicine ($C_{22}H_{35}O_4$) for pretreatment. Pretreatment in colchicine lasted for 5 hours, at the end of which the root tips were washed thoroughly in running tape water and placed in glacial acetic alcohol 1:3 v/v for 24 hours for fixation. After fixation, the root tips were hardened in absolute ethanol for one hour and washed in 30% ethanol for 3 hours before storing in 70% ethanol in the refrigerator at 4^oC until required for use.

2.3.3. Hydrolysis, Staining, and Squashing

The root tips were picked from the 70% ethanol using a clean pair of forceps into a test tube containing distilled water. After 15 minutes of repeated changes in distilled water, the root tips were rinsed in two quick changes: 1 NHCL, first in cool, followed by warm 1 NHCL. The root tip was then hydrolyzed in 1 NHCL for 8 – 10 minutes in a water bath regulated at 60^0C (Sheikh & Maher, 2007). On removal from the water bath, the root tips were rinsed again in cold 1NHCL to arrest the process of hydrolysis and again washed in distilled water for another 15 minutes. At the end of the last washing time, the root tips were sufficiently softened for squashing. Squashing was done by placing one root tip at a time on a clean, dry glass slide using a pair of pointed forceps. The young meristematic portion was cut out to allow for the discarding of the older portion. One drop of 2% acetic orcein ($C_{28}H_{24}O_7N_2$) was added to the meristematic root tip and was allowed for 60 seconds for proper stain absorption. The stained root tip was carefully covered with a clean cover slip. Gentle taps on the coverslip using the blunt end of a mounted pin ensured proper squashing of the root tips. The whole set-up was allowed to stand for five minutes for deep staining of the chromosomes to take place. The slide was mounted and viewed, first with the X10 objective of an optical binocular research microscope. Slides with good metaphase stages containing well-spread chromosomes were first drained of excess stain and then sealed using Ladies white vanish. When the seal dried, a light microscope coupled with a digital camera was used for observation, 200 cells were scored per slide, and photographs of the cell were made.

2.4. Statistical Analysis

The computer software motif images plus 2.0 were used to capture, organize, and analyse data from the microscope. A descriptive statistical function was employed in the preliminary analysis of data. The percentage of aberrations was analyzed using analysis of variance (ANOVA)

3. Result and Discussion

The potential cytotoxic and mutagenic effects of the plant extracts, root tip growth inhibition, effective concentration, and root cell morphology were identified by analyzing the percentage of aberrations. In this study, the mutagenic and mutagenic ability of *Azanza garckeana* on the root tip of *Allium cepa* were evaluated, and the result is presented as follows:

3.1. Maximum and Minimum Inhibitory Effects (EC50) on Allium Cepa Assay

There is an inhibitory effect of the concentrations of each extract on the root lengths of exposed *A. cepa* seedlings. The EC50 (Effective concentration resulting in the inhibition by 50% of the root length of the untreated *A. cepa* seedlings) was determined for each extract using a graph to indicate the maximum and minimum EC50 (Effective concentration). The EC ranged from 10 g/ml with a root length of 3 cm for *Azanza garckeana* leaf extracts and 10g/ml with a root length of 2.5 cm for *Azanza garckeana* fruit extracts. The extracts showed a decline in meristematic growth compared to the control as the concentrations of the extracts increased. As shown in (figure 1) below. This observation agrees with the work of Udo *et al*. (2014) and Tedesco *et al*. (2012), which reported the cytotoxic effect of different plant extracts. Comparatively, the effective concentration (EC50) (figures 1 & 2) showed that the plant's extract was most cytotoxic with an indication of a mitodepressive effect on the meristematic growth of the *A. cepa* root. This is also corroborated by the report of Akinboro and Bakare (2007), which evaluated the effect of different extracts of *A. indica, M. lucida, C. citratus, M. indica, C. medica, and C. papaya* on *Allium cepa* root.

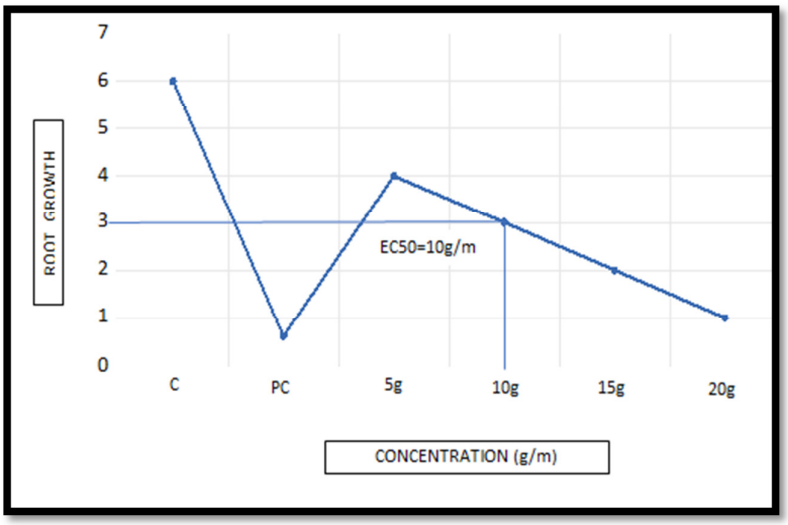

Figure 1: Inhibitory Effect of Different Concentrations (G/M) of Azanza garckeana Leaf Extract on Allium Cepa Root Growth

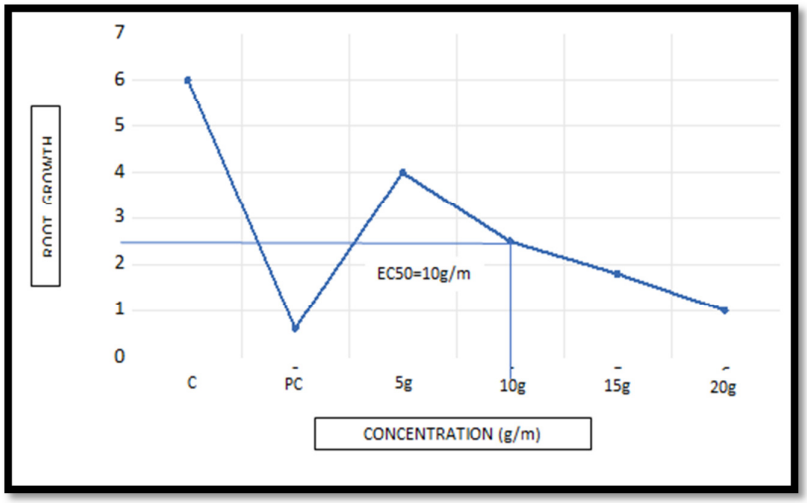

Figure 2: Inhibitory Effect of Different Concentration (g/ml) of Azanza garckeana Fruit Extract on Allium Cepa Root Growth

Conc g/ml cell	Total cell scored	TNC D	Chromosomal aberration PP	L	AB	D	VM	BN	NL	F	CG	MN	US	TNAC	% of aberration cells
NC	200	180	-	-	-	-	-	-	-	-	-	-	-	0	0[a]
PC	200	154	7	3	2	3	4	0	5	6	2	3	10	45	22.5[bc]
5g	200	173	-	-	-	-	-	-	-	-	-	-	-	0	0[a]
10	200	161	1	6	6	4	3	3	-	6	6	3	4	42	21[b]
15	200	148	8	7	5	6	4	6	4	8	5	6	6	65	32.5[cd]
20	200	142	10	6	6	7	-	8	-	10	11	5	8	71	35.5[d]

Table 1: Chromosomal Aberration Observed in the Root Cells of A. Cepa Treated with A. Garckeana Leaf Extract

PP = Polyploidy, D= dissolution, V= vagrant metaphase, BN= Binucleate, L = Laggard, NL = Nuclear lesion, F= Fragmentation, CG= Chromosome gap, AB = Anaphase bridge, MN= Micronuclei, US= unequal separation, TNCD= Total number of dividing cell, TNAC= Total number of aberrant cell.

The chromosomal aberrations observed in the root tip cells of *A. cepa* treated with the leaf extracts of *A. garckeana* in table 1 above shows that 10g is statistically significantly different from 5g (P = 0.026) and negative control, 15g is significantly different from 5g (P = 0.000) and 10g (P = 0.026). However, as the concentration increased to 20g, there was a significant difference in the percentage of aberrations from 5g (P =0.0000) to 10g (P = 0.005), but not significantly different from 15g (P =0.0553).

Conc g/ml	Total cell scored	TNDC	Chromosomal aberration PP	L	AB	D	VM	BN	NL	F	CG	MN	US	TNAC	% of aberrant cells
NC	200	180	0	0	0	0	0	0	0	0	0	0	0	0	0[a]
PC	200	154	7	3	2	3	4	0	5	6	2	3	10	45	22.5[c]
5	200	172	-	-	-	-	-	-	-	-	-	-	-	-	0[a]
10	200	170	1	1	0	5	2	1	0	1	1	2	3	17	8.5[ab]
15	200	150	6	4	3	4	2	0	3	0	5	2	8	37	18.5[b]
20	200	148	9	3	5	2	0	6	6	1	0	4	11	47	23.5[c]

Table 2: Chromosomal Aberration Observed in the Root Cell of A. Cepa Treated with Azanza Garckeana Fruit Extracts

PP = Polyploidy, D= Dissolution, V= Vagrant metaphase, BN= Binucleate, L = Laggard, NL = Nuclear lesion, F= Fragmentation, CG= Chromosome gap AB = Anaphase- bridge, MN= Micronuclei, UE= unusual separation, TNCD= Total number of dividing cell, TNAC= Total number of aberrant cell.

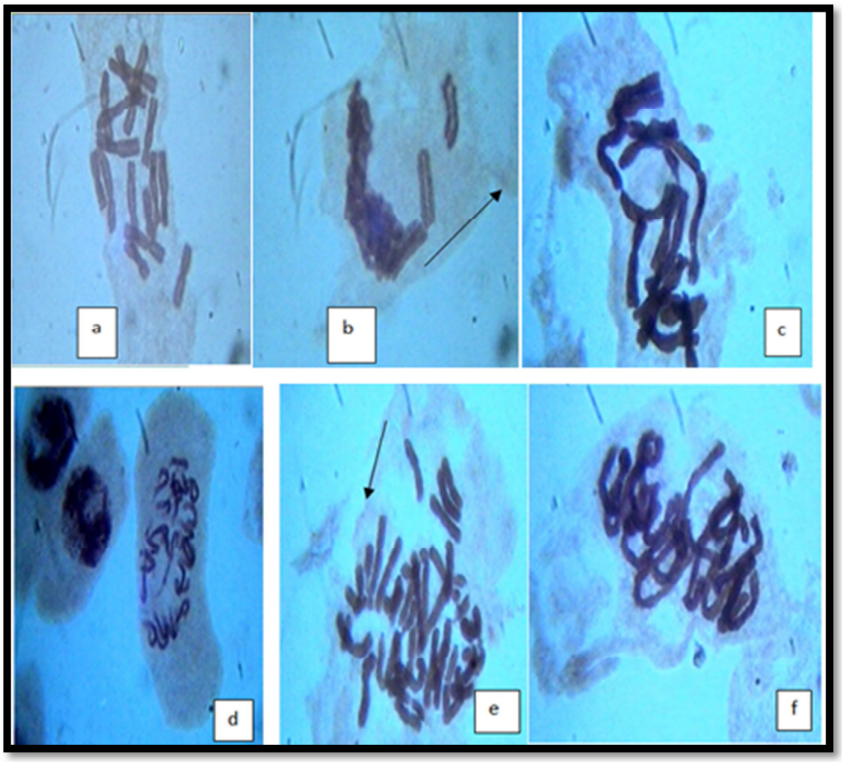

Figure 3: Macrograph Showing Different Types of Chromosomal Aberrations
(a) normal cell with 2n=16 (b) Stickiness (c) Chromosome gap(d) Nuclear lesion (e) Polyploidy (f) vagrant metaphase

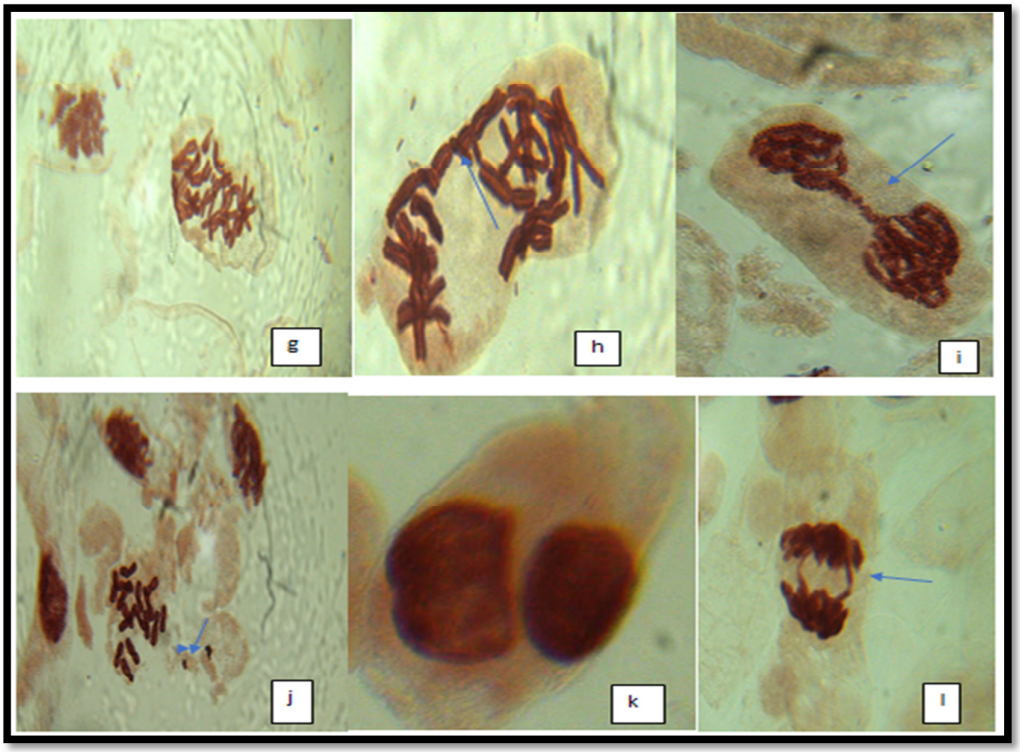

Figure 4
(g) Dissolution (h) Laggard, (i) Anaphase Bridge with dissolution,
(j) Fragmentation, (k) Binucleate cells, (l) Anaphase Bridge

The table above shows the results of chromosomal aberration observed on the root tip cell of *A. cepa* treated with *A. garckeana* fruit extracts, in the table above. Although the percentage of aberrations at 10g/10% concentration is higher than 5g there is no statistical significance (P =0.101). 15g is significantly different from 5g (P =0.101) but not significantly different from 10g (P =0.055) and 20g (P =0.331) and positive control (P =0.845). These results are in agreement with (Ganachari *et al*., 2004; Oyenke & Ayomide, 2013), who reported that external stimuli mutagens (synthetic and biological compounds) which are usually on phytoconstituents in plant or synthetic chemical compounds (monosodium glutamate) could block cellular progress in one of the phases of the cell cycle or cell division (mito-inhibition) resulting in chromosomal aberrations which include chromosome and/or chromatid fragments, interchromatin or sub chromatid connections, nucleoplasmic bridges, heteromorphic chromosomes, dicentric or ring chromosomes, and micronuclei which are observed as c-mitosis, bridges, vagrants, sticky and attached chromosomes, multipolar anaphase (Azam-Ali *et al.,* 2012). Also, these aberrations could occur due to spindle failure orchestrated by the interaction of phytochemical constituents with the spindle apparatus (Ihegboroa *et al*.,2020). In addition to the chromosome fragments, stickiness was also observed, and in general, it was possible to observe an increase of different abnormalities as the plant extracts concentration increased and a decrease in normal cells. A strong cytotoxic and mutagenic effect of plant extracts was observed, supported by the great occurrence of sticky metaphases, leading to cellular death. This is in agreement with Fernandes *et al*. (2007), which reported that the cytotoxicity levels of an agent can be determined by increases or decreases in the percentage of aberrations. Therefore, the progressive increase in aberrant cells as the concentrations increase significantly compared to negative control indicates inhibition in cell proliferation (by the action of plant extracts or chemical substances) (Fernandes *et al*.,2007).

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