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Musk Melon Root Extract (*Cucumis Melo* L.) Inhibits Mitosis and Growth of Meristematic Cells in the *Allium Cepa* Essay through Chromosomal Abnormalities

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Abstract- *Background:* The Musk melon, *Cucumis melo*, is an essential herbaceous plant species in Cameroon for its nutritional and ethnomedicinal values. Thus, roots of this plant are not edible but are used in treating many local ailments. To date, there has been no report on the cytotoxicity and genotoxicity of the roots of Musk melon. Therefore, this study was to investigate the potential cytotoxic and genotoxic effects of aqueous extracts of the roots of *C. melo* using the *Allium cepa* assay. Allium bulbs were treated with six concentrations (0 µg/ml, five µg/ml, ten µg/ml, 20 µg/ml, 30 µg/ml, and 40 µg/ml) of aqueous extract of the roots of *C. melo* for 96 hours and distilled water was used as the control.

Results: The results showed significant dose-dependent ($p<0.05$) inhibition of sprouting and growth of roots as well as mitodepressive effects on cell division in *A. cepa* root tip cells treated with aqueous extract of the roots of *C. melo*.

Keywords: *Cucumis melo L., aqueous extract, mitotic index, chromosomal abnormalities, Allium cepa L., cancer cell model, mitodepressant.*

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Results: The results showed significant dose-dependent ($p<0.05$) inhibition of sprouting and growth of roots as well as mitodepressive effects on cell division in *A. cepa* root tip cells treated with aqueous extract of the roots of *C. melo*. The results also revealed a significant increase in the dose-dependent frequency of chromosome aberrations (Anaphase bridges, laggards, disorientation, sticky metaphase, and binucleation) in the *A. cepa* root tip cells.

Conclusions: This study revealed that *C. melo* roots have a clastogenic effect on the root tip cells of *A. cepa*. The following research will test this extract on a few cancers induced in Wistar rats.

Keywords: Cucumis melo L., aqueous extract, mitotic index, chromosomal abnormalities, Allium cepa L., cancer cell model, mitodepressant.

I. Background

The Musk melon (*Cucumis melo* L.) is an annual herbaceous plant species native to intertropical Africa and belongs to the family Cucurbitaceae. The fruit, which is fleshy and edible, is gaining productive prominence in Cameroon, especially the North West, West and, Southwest Regions, because of its nutritional and ethnomedicinal values. Melons are naturally low in fat and sodium, have no cholesterol, and provide many essential nutrients such as potassium, in addition to being a rich source of beta-carotene and vitamin C [1, 2]. Chemical analysis has shown that *C. melo* is rich in moisture, carbohydrate, dietary fibers, minerals, carotenoids, folate and flavonoids such as B-carotene lending, xanthin cryptoxanthin, and phenolic compounds. Phenolic compounds are volatile. They are biosynthetically derived from fatty acids, carotenoids, amino acids and terpenes, while non-volatile constituents include constituents such as B-carotenes, flavonoids, carbohydrates, linoleic acid, acid a-linolenic, glycolipids, phospholipids, amino acids, phenolic compounds. glycosides [3, 4, 5, 6]. The total bioactive components of *C. melo* have important health values. Traditionally, *C. melo* is used to treat kidney stones, flatulence, leprosy, fever, jaundice, diabetes, obesity, cough, bronchitis, ascites, anemia, constipation and other abdominal disorders [7, 4, 8]. These total compounds have medicinal value, since the fruits and roots of C. melo are consumed for their therapeutic value [4]. In Cameroon, based on traditional knowledge that has accumulated over centuries, the leaves, the pulp of fruits, and the seeds of C. melo are eaten for their medicinal properties, while the roots are only used for medicinal purposes. The investigating the cytotoxicity and genotoxicity of the sources of *C. melo* will serve as a measure of safety for its continued use for medicinal purposes.

II. Methods

a) Origin of Plant Material and Preparation of Extracts

In this study, the aqueous extract of the roots of *Cucumis melo* was used as the test substance, while the root of *Allium cepa* was used as the test system. The roots of *C. melo* were obtained from a farm in Balessing in the West Region of Cameroon. At the same time, the Violet Galmi variety of onion (*A. cepa* of pure line L78 to eliminate individual variations) was provided to us by the Institute of Agricultural Research for Development (IARD) of Maroua (Far North Region, Cameroon). The roots of *C. melo* were washed in clean water, chopped into small pieces, and then dried in an oven at 48°C for two days. Therefore, dry and brittle roots were ground and used to prepare the aqueous solution using the method of [9]. The *Allium cepa* tests were held at the Applied Biology and Ecology Research Unit (URBEA) Laboratory according to the general description of [10].

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Thus, viable bulbs of *A. cepa* were placed in small transparent disposable cups with their basal ends dipping into various concentrations of the aqueous extract of *C. melo* roots (0 µg/ml, five µg/ml, ten µg/ml, 20 µg/ml, 30 µg/ml and 40 µg/ml and germinated at room temperature (25 - 30°C) for 96 hours. The control group of onion bulbs was dipped into distilled water (0 µg/ml). For each treatment, five bulbs were used per concentration of the test samples.

b) Macroscopic Examination

The onion bulbs were analysed for number of roots germinated and the length of the roots. Total number of roots grown per onion bulb was counted and recorded. To determine root length, ten cores were randomly taken from each treatment and measured with the help of a hardened stainless-steel digital caliper 150mm.

c) Cytogenetic Analysis

Meristematic root tips were obtained from the roots used for the measurement of lengths. The squashes method was prepared as suggested by [11] using 2% aceto-orcein. The chromosome smears thereof were used to determine the Mitotic Index (MI) and the presence of Chromosomal Aberrations (CA) induced by the aqueous extracts of the roots of *C. melo*. Five replicates were performed for each treatment, and scoring was done from the ten sources of each replicate. A minimum of 200 mitotic cells were counted from each slide. Mitotic indices (MI) were calculated for each treatment according to the formula: Number of dividing cells/Total number of cells observed×100. Chromosomal abnormalities were scored in the mitotic cells, and the results are shown in the tables and figures, while the most frequent chromosomal abnormalities are shown in photomicrographs.

d) Statistical Analysis

The data expressed as means ± standard deviation and one-way ANOVA and Tukey's post-test (HSD) were applied to evaluate the significances ($\alpha<0.05$) of between-treatment differences in percentage germination, root length, mitotic index, and frequency of chromosome and nuclear abnormalities. Python software version 3.2, and Pandas packages, were used under the MacOSX operating system.

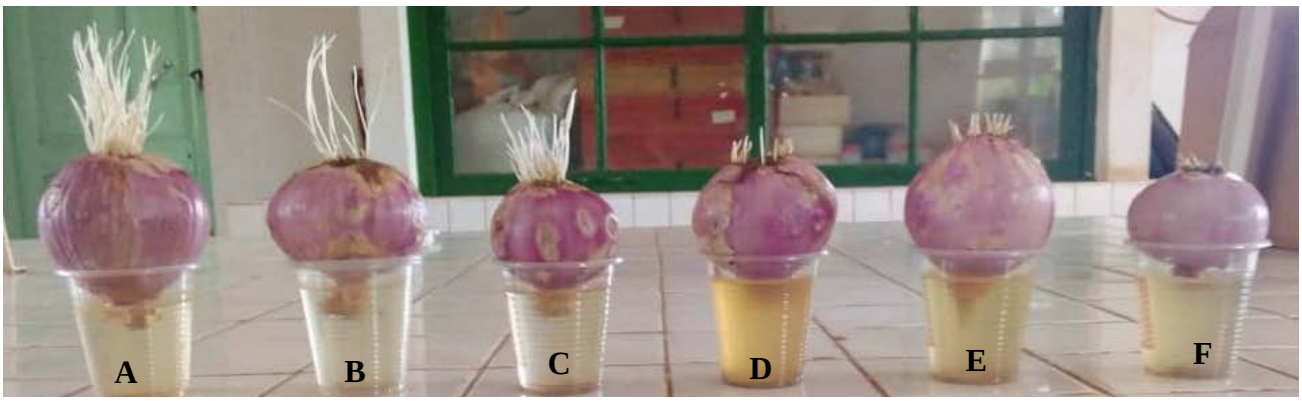
III. Results and Discussions

During the present study, the potential genotoxicity of the aqueous extract of the roots of *C. melo* was evaluated with the *A. cepa* essay. This cytotoxicity and genotoxicity were by analyzing two macroscopic parameters that included the number of roots sprouted and the lengths of the bases, as well as microscopic parameters such as the frequencies of MI and Cas in A. cepa cells plant growth, is an irreversible increase in size resulting from mitotic cell division and cell enlargement. The mitotic process also involves tight control and coordination of proliferative activity and growth in meristematic and differentiated tissues [12]. It is considered that, when the proliferative activity (mitosis) is disrupted, growth inhibition follows. In addition, disruption of mitosis could be the failure to respond appropriately to the stressor signal present in its environment [13]. During this study, the stressor signal was provided by the aqueous extract essential the roots of *C. melo* affected root growth by affecting mitosis in the meristem of the root tip. Therefore, follows that plant root growth inhibition is an indicator of macroscopic cytotoxicity [14, 15, 2].

The mean number of roots and mean lengths of the roots of onion bulbs grown in control (distilled water) and the different concentrations of aqueous extracts of *C. melo* are shown in Table 1. The results obtained for the further concentration of aqueous extracts (5 µg/ml, ten µg/ml, 20 µg/ml, 30 µg/ml, and 40 µg/ml) indicated that the roots grew less when treated with various concentrations of aqueous extracts of *C. melo* (102.10±4.18%, 94.44±5.00%, 91.14±2.02%, 59.02±1.04% and 35.11±0.40% respectively) than in the control group (113.15±6.10) (Table 1). Inhibition of root growth was, therefore, concentration-dependent and was statistically significant ($P < 0.05$) at the test concentrations. For all concentrations of the aqueous extract of *C. melo*, restricted root germination implied toxicity. Mitotic activity expressed here as MI was the first parameter used to evaluate the cytotoxicity of the aqueous extract of *C. melo*. Table 3 carries the MI levels for the control and each treatment concentration. As per the data in Table 3, the cytotoxicity levels were observed to increase with a decreased rate of MI. It is evident from this data (Table 3) that the aqueous extract of the roots of *C. melo* reduced MI compared to the control group. However, the decrease in MI with the extract concentration was extract slight and essential there was stagnation in the mitotic division once the cells entered the Metaphase stage. The extracts reducethe number in cells moving from Metaphase to Anaphase. The data also showed that MI was positively correlated to root length, which decreased with increasing concentration of the aqueous root extracts of *C. melo*. The mean number of dividing cells was lowest in the highest concentration (40 µg/ml) of the aqueous root extract of *C. melo*. Therefore, in this study, aqueous extract of the roots of *C. melo* decreased MI of A cepa root tip cells. This decrease was significant for all treatments concentrations (5 µg/ml, ten µg/ml, 20 µg/ml, 30 µg/ml, and 40 µg/ml) when compared to the control group. These results showed that all treatment concentrations of the aqueous extract of the roots of C. melo were toxic on A. cepa root tip cells. It is suggested here that the bioactive compounds contained in the roots of C. melo have mitodepressant properties, that's to say slow down cell division in the meristematic cells of the root end of

A. cepa however, without killing them. The resumption of cell proliferation is observed when these mitodepressed roots are immersed again in distilled water. It has been variously shown that the mitodepressive effects of some plant extracts have the ability to block the synthesis of DNA and nucleus proteins [16, 17, 18] thereby reducing MI and hence plant growth. In the case of *C. melo*, bioactive substances may not have allowed the formation of spindle proteins hence reducing the movement of cells from metaphase to anaphase.

This study was conducted using chromosome aberrations to detect the clastogenic activity of the aqueous extract of the roots of *C. melo* on the meristematic cells in the root tips of *A. cepa*. A summary of information on aberrant chromosomes in dividing cells of the root tips of *A. cepa* treated with different concentrations of the aqueous extract of the roots of *C. melo* is shown in Table 3. In the meristematic cells of *A. cepa* treated with different concentrations of aqueous extract of the roots of *C. melo*, Anaphase bridges, laggards and disorientation, nuclear vacuoles, sticky metaphase and double nuclei (Fig. 2) were found and their frequencies were higher than in the control group. These chromosomal abnormalities were used to quantitatively and qualitatively evaluate the genotoxic potentials of the aqueous extract of the roots of *C. melo*. Chromosome abnormalities such as recorded in this study probably occurred due to lesions in DNA as well as chromosomal and spindle proteins that cause genetic damage [19]. The genetic damage brings about drastic changes in chromatin, spindle apparatus, and centromere thus preventing alignment at the metaphase plate, and abnormal spindle orientation. This has been shown to occur due to altered quality and quantity of kinetochore heterochromatin [20, 21]. Total chromosome aberration (CA) frequencies in the treated groups were found to be higher than in the control group, and all the differences were statistically significant ($p<0.05$). The total percentage of CAs significantly increased with the concentration of extract. Therefore, CAs were significantly dose-dependent. CA was not found in the control group. Although The frequencies of aberrant chromosomes increased after treatment, a dramatic increase was recorded with the highest concentration of the aqueous extracts of the root of *C. melo* (Table 3). The results of this study revealed that aqueous root extracts of C. melo had a clastogenic effect on the root tip cells of *A. cepa*, since all concentrations tested induced multiple chromosomal abnormalities.



A=0 µg/ml (control); B=5 µg/ml; C=10 µg/ml; D=20 µg/ml; E= 30 µg /ml; F=40 µg/ml.

Figure 1: Examples of series of onions (*A. cepa*) cultivated for 96 h in different concentrations of the aqueous extracts of *Cucumis melo*

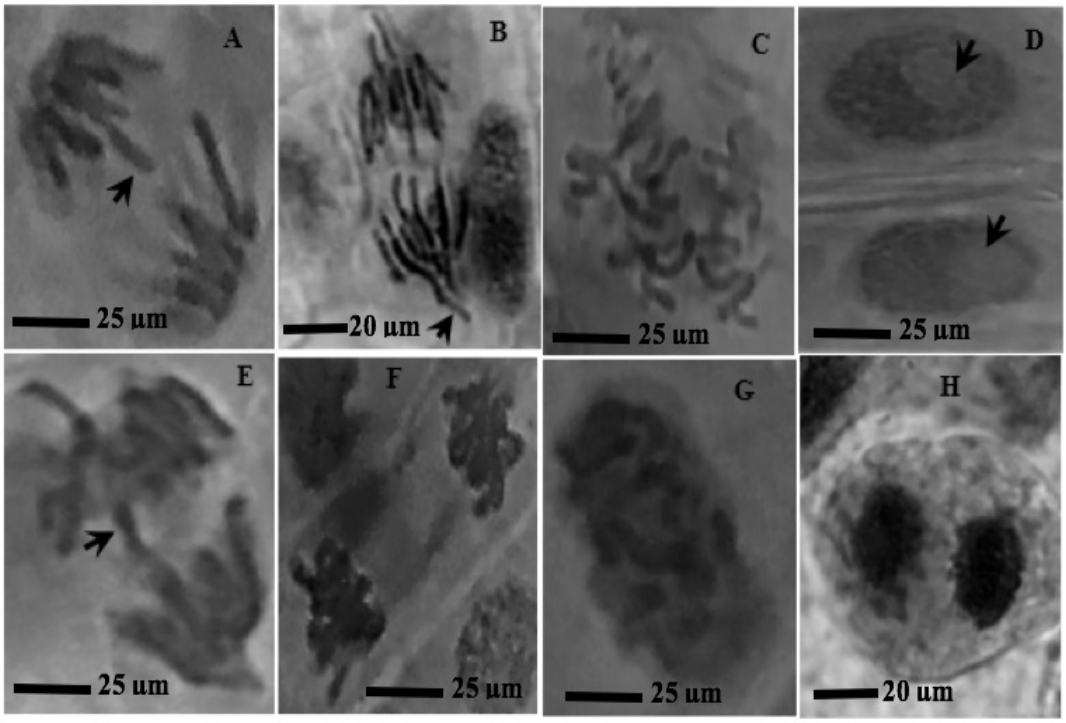


Figure 2: Mitotic and chromosomal aberrations (CA) (arrowed) induced *in Alliumcepa* root tips by aqueous extracts of the roots of *C. melo*. A and B=Anaphase laggard; C= Disoriented Anaphase; D= Nuclear vacuoles; E=Anaphase Bridge; F=Sticky chromosomes; G=Mature cell showing puff; H=binucleated cell

Table 1: Effects of aqueous extract of *Cucumis melo* on the sprouting of *Allium cepa* roots

Concentration (µg/ml)	Mean root number ± SE	% Total root sprouted of NC	Percentage inhibition	95% confidence limit
0	113.15 ± 6.10^{a}	100	0	0.000
5	$102.10\pm4.18^{*b}$	81	19	0.012
10	$94.44\pm5.00^{**cd}$	59	41	0.010
20	$91.14\pm2.02^{***ef}$	49	51	0.017
30	$59.02\pm1.04^{***gh}$	15	85	0.011
40	$35.11\pm0.40^{***i}$	3	97	0.010

*Number of trials n=5, *p<0.05; **p<0.01; *** p<0.001 significantly different from the control group (Distilled water), applying one-way ANOVA followed by Tukey's post-test (HSD). Groups that have no letters in common differ significantly.*

Table 2: Effects of aqueous extract of *Cucumis melo* on the growth of *Allium cepa* roots

Concentration (µg/ml)	Mean root length ± SE	% Total root growth of NC	Percentage inhibition	95% confidence limit
0	$5.77\pm0,5^{a}$	100	0	0.000
5	$4.57\pm0,11^{*b}$	81	19	0.012
10	$4.11\pm0,31^{*cd}$	59	41	0.010
20	$4.06\pm0,13^{**e}$	49	51	0.017
30	$3.88\pm0,26^{**e}$	15	85	0.011
40	$2.89\pm0,24^{**f}$	3	97	0.010

*Number of trials n=5, *p<0.05; **p<0.01 significantly different from the control group (Distilled water), applying one-way ANOVA followed by Tukey's post-test (HSD). Groups that have no letters in common differ significantly.*

Table 3: Cytogenetic analysis of *A. cepa* root tips exposed to different concentrations of aqueous extracts of the roots of *C. melo*

Conc. (µg/ml)	Total cells examined	% Prophase Index	% Metaphase Index	% Anaphase Index	Mitotic Index (MI)	Anaphase					
						Bridges	Laggards	Disoriented	Nuclear vacuoles	Sticky chromosomes	% Total abnormalities
0	454	15.80	14.20	14.40	45.40±1.18[a]	0	0	0	0	0	0[a]
5	439	15.50	15.00	13.40	43.90±1.53[*b]	5	3	6	2	4	3.83±1,47[*b]
10	424	10.40	19.80	12.20	42.40±1.81[*c]	6	5	8	2	6	5.16±2,04[*cd]
20	426	5.70	25.90	11.00	42.60±1.34[***c]	9	6	12	5	9	7.50±3,01[*ef]
30	452	4.60	30.10	10.50	45.20±1.06[**ce]	11	7	14	6	10	8.83±3,43[**g]
40	437	3.30	30.30	10.10	43.70±1.39[***f]	12	9	15	7	11	10.00±3,34[***h]

*Number of trials n=5, *p<0.05; **p<0.01; *** p<0.001 significantly different from control group (Distilled water), applying one-way ANOVA followed by Tukey's post-test (HSD). Groups that have no letters in common differ significantly.*

Abbreviations List
Not used

Declarations

Ethical Approval and Consent to Participate
All experimental studies on plants were complied with relevant institutional, national, and international guidelines and legislation.

Consent to Publication
We declare hereby that this work has not been published or accepted, in whole or in part, and that it is not selected for publication in another journal. All authors have approved the manuscript and agree with its submission.

Availability of Data and Material
Datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing Interests
The authors have no competing interests to declare that are relevant to the content of this article.

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Author Contributions
N.A. and S.R.A. conceived and conducted research experiments, N.A. analysed data and conducted statistical analyses, N.A. wrote the original draft article, S.R.A reviewed the manuscript.

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