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Cytotoxicity Of Rosy Periwinkle (Catharanthus roseus) Crude Extract on Onion (Allium cepa) Root Tip Cells

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Abstract — Plants have been rich sources of compounds for the development of clinically useful therapeutic agents. Catharanthus roseus has a mutagenic action due to the presence of alkaloids, making it an eminent anticancer herb along with many other therapeutic effects. In response, this research intends to establish experimental basis of the claim by investigating the cytotoxic property of the rosy periwinkle leaves extract. The crude extract of rosy periwinkle of varying concentrations of 100%, 66.67%, and 33.33% was tested against the root tip cells of onion. Pure distilled water was used as control to compare the efficacy of the extract in varying concentrations, having three replicates for each treatment. Within two weeks of experimentation, germination, cell fixation, cell staining, and microscopy was done in order to gather data and proceed to evaluation. The experimental results revealed that the rosy periwinkle leaves extract has a varying effect on its dependent variables namely: frequency of cytological aberrations and frequency of chromosomal aberrations depending on its varying concentrations. Using the One-Way Analysis of Variance at 0.01 level of significance, results revealed that only in the frequency of cytological aberration parameter did the researcher observe a significant difference, with a significance level of 0.001, the rest did not. Regardless of the majority having no significant difference, the findings still imply that the extract of rosy periwinkle leaves demonstrates a cytotoxic property against the onion root tip cells.

Keywords — Catharanthus roseus, Cytotoxicity, Rosy periwinkle, Onion root tip cells, Chromosomal aberrations, Cytological aberrations, Mutagenic action

I. Introduction

Cell division holds a vital role in all living organisms since its sole purpose is to sustain life. Throughout our existence, our cells are renewed by a process of division and duplication that is necessary for the regeneration, replication, and replacement of worn out cells. When you get a cut in your skin, cells divide to close the wound. When an egg cell gets fertilized by a sperm, cell division ends up producing an embryo which grows into a new organism. There is no reproduction without cellular division. Thus, growth wouldn't be possible, therefore making it a fundamental event in cellular biology. Cellular division is probably the most important physiological event in all biology, and it is basically the strategy of life to overcome death (Kuras et al., 2006). However,

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cases nowadays include the abnormal and rapid cell growth resulting to serious conditions such as cancer, which is caused by the overgrowth of abnormal cells in a certain body location that may spread all throughout the body and may be fatal if left untreated.

Cancer is the second leading cause of death globally and is estimated to have risen to 18.1 million new cases and 9.6 million deaths in 2018 according to a report released by the International Agency for Research on Cancer (IARC). Globally, about 1 in 6 deaths is due to cancer (World Health Organization, n. d.).

The majority of cancers are due to genetic mutations or heredity, environmental and lifestyle factors, and infections. But in some cases, the causes of cancer remain unclear for scientists and doctors.

On the other hand, several medications have been established to treat medical complications like cancer. One of these is the chemotherapy treatment, in which strong anticancer chemicals are used to remove, damage, or kill the cancer cells in a certain area. However, financially challenged people may find this treatment costly, leading to the disconsolation of the said disease, which obviously does endanger life.

Upon making some observations and several researches, the researcher found out that there is a certain plant that contains extracts which are postulated to have anticancer properties, the rosy periwinkle plant (*Catharathus roseus*). According to Latha et al. (2017), *Catharanthus roseus* produces vinca alkaloids which are well known for their anti-cancer properties. Some of the alkaloids are vinblastine, vincristine, vinorelbine, and vindesine. Anti-cancer drugs derived from *Catharanthus roseus* act as inhibitors of tubulin by binding to α/β- –tubulin. This prevents its association into microtubules which provide cells with both the structure and flexibility they need to divide and replicate. Microtubules are the building blocks of protein and are vital for the proper functioning of the mitotic spindle in a mitotic cell division. Vinca alkaloids are known as mitotic spindle poisons, as they inhibit further assembly of the spindle forms from microtubules, thereby inhibiting mitosis in cell cycle.

Since the extract of rosy periwinkle plant is believed to contain potential chemicals that can inhibit mitosis cell cycle (Taylor & Fransworth, 1975), the researcher wants to test its cytotoxicity by using onion (*Allium cepa*) root tip cells as subject to find out if it can possibly inhibit its process of cell division. Additionally, the researcher would want to knows the effect of rosy periwinkle crude extract in varying concentrations on onion root tip cells in terms of frequency of cytological aberrations; and frequency of chromosomal aberrations.

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Literature Review

Rosy Periwinkle Plant

Catharanthus roseus, commonly called as Rosy periwinkle or Madagascar periwinkle in foreign countries and known as tsitsirika in the Philippines, is an erect to spreading tender perennial in the dogbane family typically mounding 6-11 inches tall. As an ornamental plant, it is appreciated for its hardiness in dry and nutritionally deficient conditions, popular in subtropical gardens where temperatures never fall below 5–7°C. It is a warm season bedding plant in temperate gardens. Rosy periwinkle is noted for its long flowering period, throughout the year in tropical conditions, and from spring to late autumn, in warm temperate climates. Full sun and well-drained soil are preferred for it to grow. The variation in flower colour are from white, mauve, peach, scarlet, and reddish orange (Flora of China Editorial Committee, 2015).

Chromosomal Aberration

Many types of chromosomal abnormalities exist, but they can be categorized as either numerical or structural. Numerical abnormalities are whole chromosomes either missing from or extra to the normal pair. Structural abnormalities are when part of an individual chromosome is missing, extra, switched to another chromosome, or turned upside down.

A chromosomal disorder, disorder, anomaly, aberration, or mutation is a missing, extra, or irregular portion of chromosomal DNA. It can be from a typical number of chromosomes or a structural abnormality in one or more chromosomes. Chromosome mutation was formerly used in a strict sense to mean a change in a chromosomal segment, involving more than one gene. A chromosome anomaly may be detected or confirmed in this manner. Chromosome anomalies usually occur when there is an error in cell division following meiosis or mitosis. There are many types of chromosome anomalies. They can be organized into two basic groups, numerical and structural anomalies. (Rieger et al., 1968).

Numerical disorders or aneuploidy occurs when an individual either is missing a chromosome from a pair (monosomy) or has more than two chromosomes of a pair (trisomy, tetrasomy, etc.) (Celik & Aslanturk, 2010). An example of trisomy in humans is down syndrome, which is a developmental disorder caused by an extra copy of chromosome 21; the disorder is therefore also called trisomy 21. Having an extra copy of this chromosome means that individuals have three copies of each of its genes instead of two, making it difficult for cells to properly control how much protein is made. Producing too much or too little protein can have serious consequences. Genes on chromosome 21 that specifically contribute to the various symptoms of Down syndrome are now being identified. The frequency of Trisomy 21 has been determined to be a function of advanced maternal age.

In microscopy, chromatin aberrations such as breaks and polar deviations, bridges in the anaphase, vagrant cells, laggings, stickiness, polyploidy, abnormal spiralisation, multipolarity, and

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abnormal kinetics are used in determining and testing drug safety, or commonly, cytotoxicity (Nefic et al., 2013).

Cytological Aberrations

The analysis of different cytological aberration types, in all phases of the cell cycle, enables a better investigation of the effects of a cytotoxic chemical or mutation, concerning its clastogenic, aneugenic and tubergenic effects. Breakages may occur and subsequent inhibition of repair mechanisms may lead to base mismatch, mutation and cytological aberrations such as fragment chromosomes and DNA breaks. Examination of anaphase chromosomes for fragments and bridges is useful for obtaining clastogenic activity. The presence of dicentric chromosomes and unequally exchanged chromatids undergoing translocation has been reported to be responsible for bridges in the anaphase. Cytological abnormalities in the cell includes anaphase bridges, chromosome lagging and stickiness, abnormal deviation and fragmented binucleated cells (Nefic et al., 2013).

Cytotoxicity

Cytotoxic refers to a substance or process which results in cell damage or cell death. The prefix "cyto" refers to cell and "toxic" to poison. Cytotoxic agents can kill cells in several ways. They may harm the cell so that its cell membrane is weakened, and the cell explodes or they may interfere with cell division so the cell stops growing and dividing. Treating cells with the cytotoxic compound can result in a variety of cell fates (Nefic et al., 2013). The cells may undergo necrosis, in which they lose membrane integrity and die rapidly as a result of cell lysis. The cells can stop actively growing and dividing (a decrease in cell viability), or the cells can activate a genetic program of controlled cell death, more commonly known as the process of apoptosis.

Cytotoxicity assays are widely used by the pharmaceutical industry to screen for cytotoxicity in compound libraries. Researchers can either look for cytotoxic compounds, if they are interested in developing therapeutic drugs that target rapidly dividing cancer cells, for instance; or they can screen "hits" from initial high-throughput drug screens for unwanted cytotoxic effects before investing in their development as a pharmaceutical product.

In the study conducted by Siddiqui et al. (2010), “Cytotoxic Activity of Catharanthus roseus Crude Extracts and Pure Compounds against Colorectal Carcinoma Cell Line” the researchers made use of the methanolic chemicals from the crude extracts of Catharanthus roseus as a cytotoxic chemical agent to colorectal carcinoma cell line. The preliminary cytotoxicity study demonstrated dose independent cytotoxic activity of the methanol extract of Catharanthus roseus when screened against HCT-116 colorectal carcinoma cell line, nhexane, chloroform and methanol fractions also showed dose independent cytotoxic activity with chloroform fraction showing the highest activity. Water fraction showed a minor cytotoxic activity. Thus, the methanolic chemicals of the crude extracts of Catharanthus roseus played a significant effect on colorectal carcinoma cell line.

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In another study conducted by Ilbas et al. (2011) entitled "Cytotoxicity of Aloe vera gel extracts on Allium cepa root tip cells" it made use of distilled water (dH2O) for the germination of the roots of the onion. This method of water germination was used by this paper's researcher. Continuing to the study of Ilbas et al., it was also stated that since the Allium cepa cell cycle is completed in 24 hours, the application process was carried out at 24 and 48 hours for investigating the mitotic and phase indexes in 2 consecutive cell cycle periods. For the first 24 hours, Allium cepa were exposed to the different treatments of different concentrations. After the experimentation involving the germination and application of treatment, it was then followed by fixation and staining of the root tip cells. The root tip cells were fixed, stained, and examined using a compound microscope. The treated roots were rinsed in distilled water and cut into segments of 1-2 cm length from the tips and fixed in pure glacial acetic acid (45%) for 30 min at room temperature. After the fixation process, it was then stained with 2% aceto-orcein solution for 3-6 min. Root tips (1-2 mm) were cut into tiny pieces and covered with a cover slip. The cells were subsequently squashed by knocking with a blunt end of a pencil and pressing slightly down with the thumb. Excess liquid was sucked up by a piece of blotting paper. Slides were scanned to investigate the different stages of mitosis. The same process of experimentation namely: the germination, application, fixation, and staining were followed by the researcher in conducting this research study, however different extracts and chemicals were used.

Data gathered from the experiment were analyzed using one-way analysis of variance, in which the researcher of this paper also utilized. Duncan's multiple range tests was then used to determine which mean values were different at the 5% level of significance. Their results showed that all tested concentrations of Aloe vera gel extracts, except for 5%, caused a significant inhibition in mean root growth rate. In the control, the mean root length decreased by 65% after the application of 30% gel extracts. It was reported that the number of dividing cells in Allium cepa cells decreased parallel to the inhibition in root growth rate. The results of this study indicated that the cytotoxic effect of Aloe vera gel extracts depend on their concentration rather than the time period, with even the low doses demonstrating a considerable rate of inhibition in root growth rate. Moreover, the detractive effect of Aloe vera extracts on mitotic index *of Allium cepa* shows that it has a cytotoxic effect on root tip cells.

II. Methodology

A. Collection and Preparation of Materials and Equipment

Onion Bulbs. Four (4) pieces of onion bulbs were purchased in a supermarket. Each onion was weighed for exactly 1g using an electronic weighing scale for it to be purchased. All the chosen onions were purchased, washed, and placed together in a bowl.

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B. Germination of Onion bulb

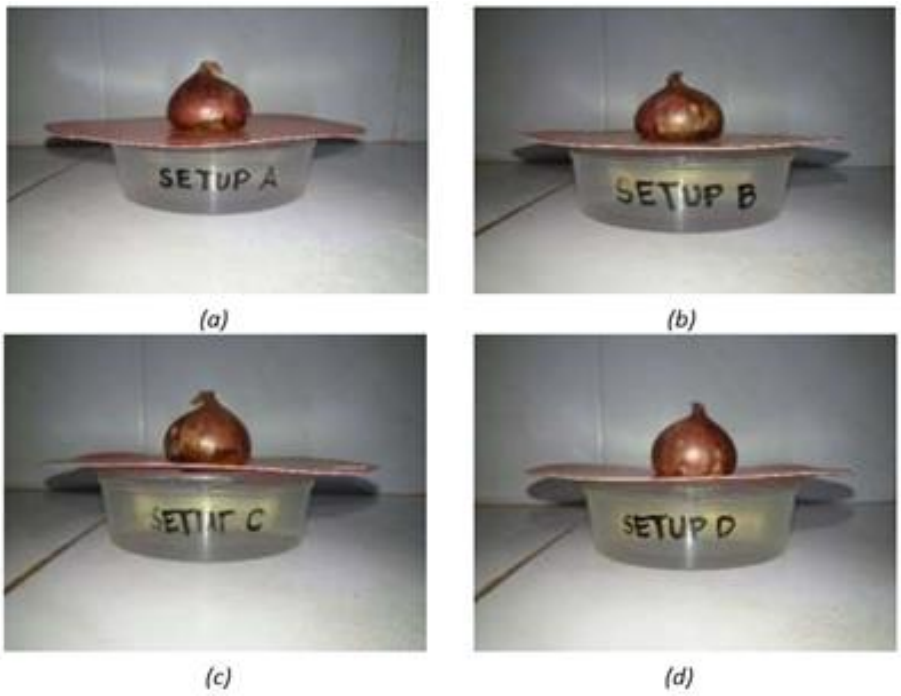
Four 200 mL round plastic containers were prepared on top of a table. The containers were marked as setups A, B, C, and D individually using a black marker pen and paper tape. 150 mL of distilled water was dispensed in each container.

Each onion bulb was numbered using the Hindu Arabic numbers from 1 up to 4. The researcher used the lottery method to choose which among the onions were included in each setup, moreover, having one onion in each setup.

After the onions were grouped, the researcher measured the diameter of the plastic container's mouth, and made four circles from the cardboard with a diameter measuring 1 inch greater than that of the diameter of the plastic container's mouth with the help of a compass for drawing, and scissors for cutting. From the four circles made from the hard board, a center hole (with a diameter lesser than that of each onion's diameter in every group) were cut to make sure that if the bulb is inserted between the holes, one-third of the bulb is immersed in the distilled water.

Then, the onion bulbs were germinated in distilled water at room temperature for three sessions with 11 hours light/ 13 hours dark photoperiod, totaling to 24 hours per session.

Figure 1.
(a) Preparation of setup A.
(b) Preparation of setup B.
(c) Preparation of setup C.
(d) Preparation of setup D.



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C. Collection and Preparation of Rosy Periwinkle Crude Extracts

This procedure was conducted after the three sessions of onion germination.

One (1) kilogram of rosy periwinkle leaves were collected from the streets of Sicayab, Dipolog City. The collected plant parts were washed, dried, and placed inside a clean container. After air drying the plant parts, the leaves were cut into tiny shreds, minced inside a blender, and placed inside an electronic juicer to separate its extract from the residual juiceless parts. A total amount of 305 mL of leaf extracts were collected in order to proceed to the next procedure.

D. Preparation of Set-ups

Another set of four 200 mL plastic containers were prepared on top of a table. Each plastic container was marked with a corresponding letter, namely: A, B, C, and D using a black marker pen. The container marked with A was considered as Setup A, B as Setup B, C as Setup C, and finally D as Setup D. Each setup must have a total amount of 150mL of and dH_2O solution.

Setup A constituted of 100% crude extract. Setup B comprised of 66.67% crude & 33.33% dH_2O. Moreover, setup C consisted of 33.33% crude & 66.67% dH_2O. On the other hand, setup D which is the control group, contained 100% of dH_2O.

For better understanding, a table is presented below:

Setup		% concentration of crude extract	Equivalent mL of extract	% concentration of dH_2O	Equivalent mL dH_2O	Total
Experimental	A	100%	150 mL	0%	0	150
	B	66.67%	100 mL	33.33%	50	150
	C	33.33%	50 mL	66.67%	100	150
Control	D	0%	0 mL	100%	150	150

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A graduated cylinder was used for measuring the amounts needed in making the solution. For setups A, B, C, and D; each solution constituting of and dH_2O was mixed homogeneously in a beaker. After homogenizing the extracts with dH_2O of each setup, it was then dispensed to its corresponding plastic container.

E. Application of Rosy Periwinkle Crude Extract Treatments and dH_2O

After the onion roots were grown in dH_2O, the same single onion of each setup during germination is transferred to a different container containing the extracted liquid from the plant parts. It was then exposed to its corresponding concentration setups containing the pure crude extract, solutions comprising the crude extract and dH_2O, and dH_2O for three (3) cycles, 24 hours per cycle, totaling to 72 hours or three days. The manner of exposing the onion was the same as the process of germinating the roots in distilled water.

Figure 2.
Germination of onion in treatment.

Preparation of Acetic Ethanol Fixative

The researcher made use of absolute ethanol (100% ethyl alcohol) and glacial acetic acid solution as a fixative with the ratio 3:1. Forty (40) mL solution per setup was prepared. Thirty (30) mL absolute ethanol was measured with the help of a graduated cylinder and mixed with ten (10) mL glacial acetic acid, also measured using graduated cylinder. Four 40mL solutions were prepared for the fixation. The four prepared solutions were then separately dispensed in a petri dish plate, each labelled with letters from A to D.

Figure 3.
(a) Dispensing of acetic ethanol fixative into the petri dish.
(b) Labeling of prepared fixatives to its corresponding groups.

(a) *(b)*

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G. Collection of Grown Onion Root Tips

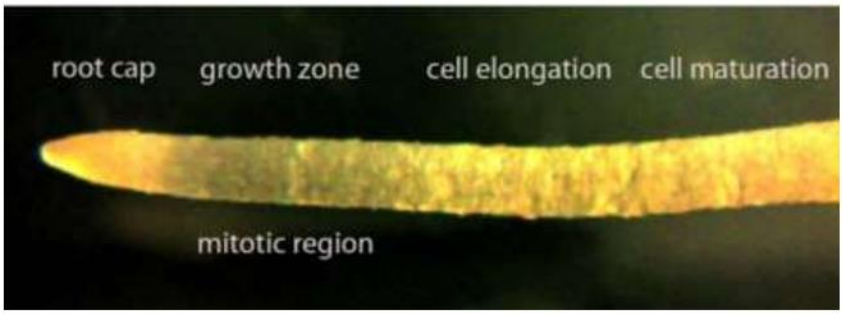
After exposing the onions to the different treatments, the grown root tips of each onion were cut and mounted on a quadrilateral-shaped watch glass separately, one (1) watch glass per setup treatment. Each root tip of each setup was numbered using the Hindu Arabic numerals with ranges depending on the number of roots per setup. A lottery method was conducted to randomly choose three onion root tips from each setup, therefore having three root replicates per group setup.

H. Onion Root Tip Fixation

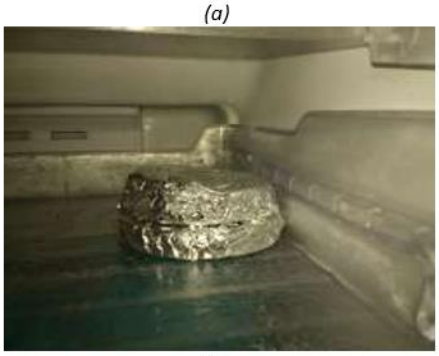
Three (3) onion root tips from each setup was used in this procedure. The root caps of each root tip are identified. From the root cap, the researcher cut at least 1 cm off. The researcher made sure that the mitotic region of the root was not included. It was then placed immediately into its designated petri dish containing the acetic ethanol fixative with the help of forceps. The same manner of preparation was followed for the root tips of the other groups. Each petri dish plate was then placed inside a refrigerator for two hours for the fixation process of the onion root tips.

For better understanding regarding to onion root tip parts, a diagram is presented below:

Figure 4.
(a) Parts of an onion root tip.
(b) Refrigeration of acetic ethanol fixatives.



Source: www.gtac.edu.au
(a)



(b)

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I. Preparation of Roots

The roots were removed from the fixative one at a time with the help of forceps. The removed roots were washed with distilled water using a watch glass. From the root cap, the researcher sliced off the 0.5 cm end portion (the pointy growing root tip) using a stainless-steel blade, making sure that the mitotic region stays and the other parts discarded. The root tips were placed on a watch glass with two (2) mL drops of 0.1 M hydrochloric acid using a dropper, enough to cover it. Using a test tube holder, the watch glass with the root tip in acid was placed on top of the flame of an alcohol lamp for 1 minute. It is important that the solution does not boil or dry out. Thus, additional 1 mL drop of hydrochloric acid will be added if it dries. Same procedure was performed for all the other setup groups. After every heating of the root tips in hydrochloric acid, each root tips is washed with distilled water.

Figure 5.
(a) Application of hydrochloric acid
(b) Application of Heat

(a) (b)

J. Staining of Onion Root Tips

After carefully washing the root tips with distilled water, each root was transferred on a glass slide using forceps. The onion roots were stained with 2 drops of Safranin stain using a dropper. It was placed on top of the flame from the alcohol lamp for one minute to allow the stain to penetrate the tissue. Excess stain was removed using a tissue paper. After removing the stain, a small drop of glycerin was dispensed on top of the prepared root tip for it to serve as the mounting medium for the coverslip. A coverslip was placed over the roots carefully to ensure that no air bubbles will enter. Little pressure was applied in squashing the roots with the coverslip in order to avoid damaging the cells. Excess liquid was sucked up using a tissue paper.

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Figure 6.
(a) Staining of cells with safranin
(b) Application of heat to the stained roots
(c) Slicing the stained onion roots thinner
(d) Squashing the onion root tips

(a) (b)

K. Microscopic Investigation

The researcher made use of the biological microscope, TBL-400 at a total magnification of 40 × 10 borrowed from the ZNNHS Laboratory. The prepared slides were mounted in the stage and secured using stage clips. First, the illumination was turned on and it was viewed under the scanner (10x) for initial focusing. To make sure that it was positioned at the center of viewing, the stage height adjustment knobs is manipulated to move the mechanical stage left or right.

Once the onion root tip cells are clearly visible, the researcher started counting manually from the uppermost left of the slide to the right, then down and from right to left, then down and from left to right, until it reached the lowermost left or right of the specimen's image under the microscope. The same sequence of procedure was followed for the other slide setups.

L. Data Collection and Evaluation

During microscopy, the researcher examined each slide. The dependent variables namely; frequency of cytological aberrations and the frequency of chromosomal aberrations were counted and listed on a tally sheet for each setup slide.

For the first dependent variable, the frequency of cytological aberrations, it was tallied according to the most frequent abnormalities observed, namely; having fragmented and apoptotic bodies, extended cells, disturbed spindle fiber and cytoplasm reduction, micronucleus in the prophase, stickiness, morphological alterations of the shape and size of the cell, and the presence of ghost cells.

On the other hand, the second dependent variable which is the frequency of chromosomal aberration was tallied according to its most frequent abnormalities namely; having bridges in the anaphase and vagrant cells. The results of each slide setup is separated from one another.

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The values of the listed data of the onion cells were then compared to the other setups. If the rosy periwinkle crude extract induced increasing frequencies of; cytological aberrations and chromosomal aberrations during the growing process, then results will claim that this extract is proven to have a cytotoxic effect on onion root tip cells.

M. Statistical Treatment

Statistical analyses were performed using the IBM SPSS Statistics 20 software package program. Data on the frequency of cytological aberrations and the frequency of chromosomal aberrations were compared using analysis of variance (ANOVA) to confirm the variability of the data and validity of results. Differences between corresponding controls and exposure treatments were considered statistically significant at significance level < 0.01, therefore having 99% confidence level.

III. Results and Discussion

Problem No. 1 *What is the effect of rosy periwinkle crude extract in varying concentrations on onion root tip cells in terms of:*

a. frequency of cytological aberrations, and

b. frequency of chromosomal aberrations?

Table 1. Comparison of the frequencies of cytological aberrations in different concentrations.

Concentrations		Frequency of the Means of Cytological Aberrations						Total
		FN	AB	EC	S	AS	GC	
A	100%	17	15	2	3	7	11	55
B	66.67%	13	7	1	2	2	8	33
C	33.33%	7	3	1	1	1	5	18
D	0%	1	1	0	0	0	0	2

Legend:
FN – Fragmented Nucleus
AB – Apoptotic Bodies
AS – Alterations in the Shape and Size
S – Stickiness
EC – Extended Cells
GC – Ghost Cells

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Figure 7. *Comparison of the frequencies of cytological aberrations in different concentration setups.*

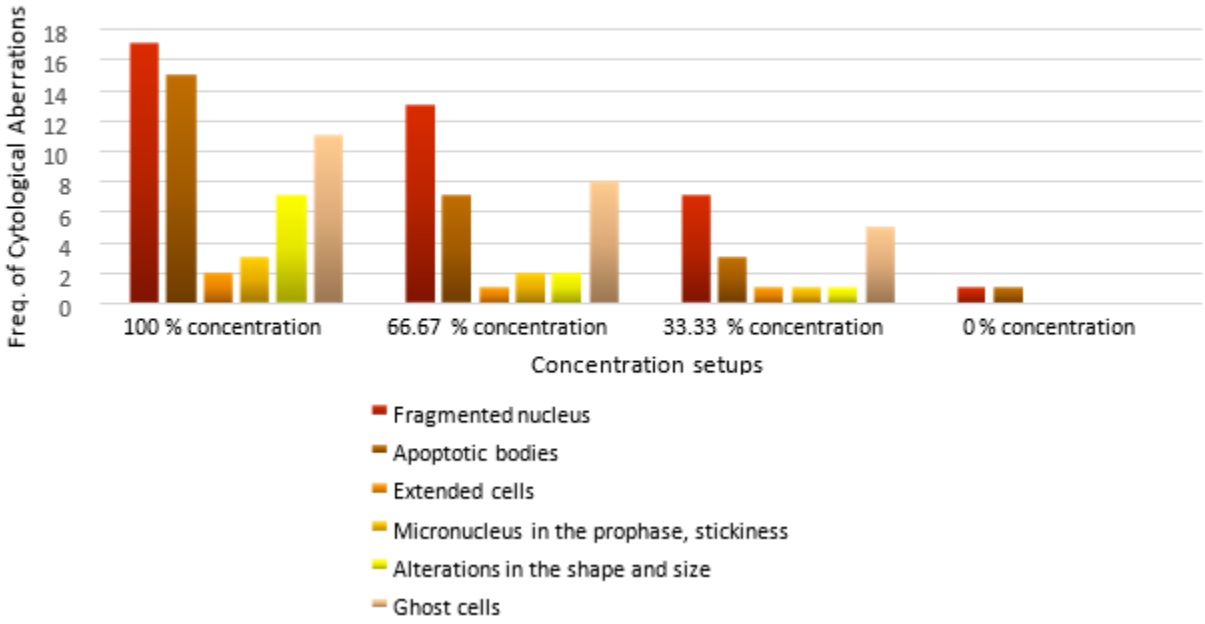

Table 1 and figure 7 show comparison of the induced effect of the rosy periwinkle leaf to the frequency of cytological aberrations in the onion specimens at different concentration setups. Setup A with a percentage concentration of 100% has a frequency count of 55. Setup B with a percentage concentration of 66.67% has a frequency count of 33. Setup C with a percentage concentration of 33.33% has a frequency count of 18. Finally, setup D, having no treatment received, has a frequency count of 2. It can be observed that the frequency of cytological aberrations increases together with the increasing concentration of rosy periwinkle crude extract, and vice versa. Therefore, rationalizing that the effect of the first setup with 100% concentration of rosy periwinkle crude extract is superior to that of the fourth setup with a concentration of 100% dH_2O, having no experimental treatment received.

The pure rosy periwinkle crude extract showed the strongest effect in the root tip cells. The observation of sticky metaphase reinforces the hypothesis of the toxic effect of rosy periwinkle crude extracts. Metaphases with sticky chromosome, loses their normal appearance, and they are seen with a sticky surface, causing chromosome agglomeration. Stickiness has been attributed to the effect of pollutants and chemical compounds on the physical and chemical properties of DNA, protein or both, on the formation of complexes with phosphate groups in DNA, on DNA condensation or on formation of inter- and intrachromatid cross links (Celik & Aslanturk, 2010).

In this study, membrane-damaged cells were observed in groups treated with pure and rosy periwinkle crude extract solutions. These results show that rosy periwinkle crude extracts over certain concentrations may cause cytotoxicity as they cause membrane damage. Siddiqui et al.,

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(2010) evaluated effects of rosy periwinkle extract on colorectal carcinoma cell line. They demonstrated that rosy periwinkle crude extract inhibited the division of cell, thus causing abnormalities. Microtubules have been implicated in cell plate formation and rosy periwinkle crude extracts can be one of the involved factors, resulting in inhibition of cytokinesis. These results are in accordance with the literature data.

Table 2.

Comparison of the frequency of chromosomal aberrations in different concentrations.

Concentrations		Frequency of the Means of Chromosomal Aberrations		Total
		bridge in the anaphase	vagrant	
A	100%	2	11	13
B	66.67%	1	7	8
C	33.33%	0	3	3
D	0%	0	0	0

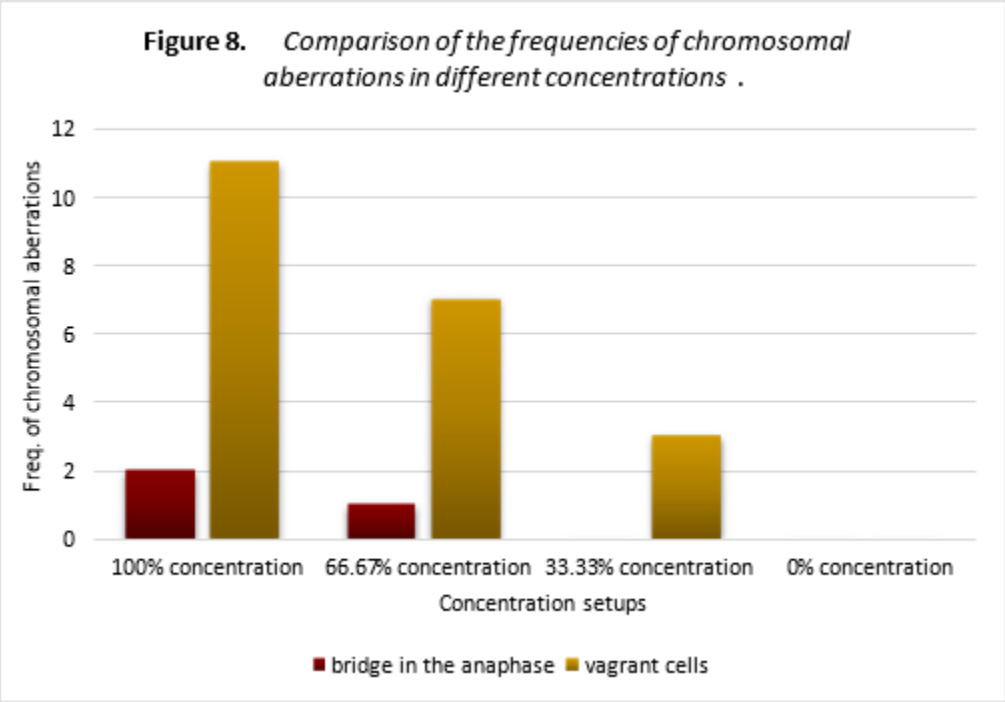

Figure 8. *Comparison of the frequencies of chromosomal aberrations in different concentrations .*

As shown in Table 2 and figure 8 the total frequencies of the four setups differ. The first setup with 100% concentration of rosy periwinkle leaves extract has a total frequency of 13. Followed by the next setup with 66.67% concentration, with a total frequency of 8. Next, the third setup with 33.33% concentration, obtained a total frequency of 3. Finally, the last setup with no experimental treatment involved, resulted to a frequency of 0. It can be observed that the frequency decreases together with the decreasing concentration of the extract. The frequencies of the four

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setups show that the ones applied with the rosy periwinkle crude extract has a greater effect compared to the setup which was never applied with the experimental treatment.

Chromosomal aberrations are changes in chromosome structure resulting from a break or exchange of chromosomal material. Most of the chromosomal aberrations observed in cells are lethal, but there are many related aberrations that are viable and that can cause genetic effects, either somatic or inherited. The presence of chromosome fragments is an indication of chromosome breaks and can be a consequence of anaphase/telophase bridges. The induction of chromosome breaks, disturbances on microtubule assembly and cellular death can be related. The results showed induction of chromosome type of aberration in the cells treated with the rosy periwinkle crude extracts. Somehow the rosy periwinkle crude extracts not only interfere with the cell cycle, but also affect chromatin organization or DNA replication, causing chromosome breaks. Frequencies of total chromosome aberrations increased significantly upon exposure rosy periwinkle crude extracts. These results are in line with the results of many research groups that examined the effects of different medicinal herbs (Celik & Aslanturk, 2010).

Problem 2: *Is there a significant difference between the effect of rosy periwinkle crude extract in varying concentrations and 0% (dH_2O) treatment on onion root tip cells in terms of the frequency of cytological aberrations and frequency of chromosomal aberrations?*

Table 3.
ANOVA General Summary Table for the Different Variables

ANOVA			
Parameters	F	Value of Significance	Decision
frequency of cytological aberrations	5.051	0.009	Reject H_o
frequency of chromosomal aberrations	1.037	0.466	Accept H_o

H_o: There is no significant difference in the effect of rosy periwinkle crude extract in varying concentrations and dH_2O treatment on onion root tip cells in terms of the frequency of cytological aberrations and frequency of chromosomal aberrations.

Table 3 presents the value of significance computed of each parameter, and the decisions whether the null hypothesis is accepted or rejected. As shown above, the values of significance of the frequency of chromosomal aberrations is higher compared to the value of alpha which is 0.01. Hence, this led to the acceptance of the null hypothesis, therefore implying that there is no significant difference between the different setups as computed statistically by a statistical software.

On the other hand, the frequency of cytological aberration parameter obtained a computed significance value of 0.009 which is lesser than the baseline which is the alpha. This implies that there is a significant difference between the varying concentrations of the rosy periwinkle crude extract against the frequency of cytological aberrations of the onion root tip cells.

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Generally, since only the parameter of frequency of cytological aberration was proven to be statistically different, statistics suggest that the different concentrations of the crude extract of rosy periwinkle has a varying effect to the cells, with the pure crude extract inducing the highest frequency of cytological aberration followed by 66.67%, and 33.33%.

The results of this study indicate that the cytotoxic effect of the crude extract of rosy periwinkle depends on their concentration, with even low dosage inducing an aberration. Moreover, the aggrandizing effect of rosy periwinkle crude extract on the frequencies of cytological and chromosomal aberration show that it has a cytotoxic effect on root tip cells. It is known that the *Allium* test shows a good correlation with mammalian test systems (El-Shabbaby, 2003; Teixeira et al., 2003). For this reason, it is possible that rosy periwinkle crude extract can have a therapeutic effect to destroy cancer cells as reported by some literatures by Ilbas et al. (2012).

IV. Conclusion

Based on the findings of the study, the following conclusions are drawn.

1. The rosy periwinkle crude extract demonstrated a cytotoxic property against the onion root tip cells as manifested by its increasing frequencies of cytological aberrations and increasing frequencies of chromosomal aberrations.

2. The potential of the rosy periwinkle crude extract varies according to its different concentration setups.

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