ISSN: 2959-6386 (Online), Vol. 2, Issue 1

Journal of Knowledge Learning and Science Technology

journal homepage: https://jklst.org/index.php/home

The Anti-Mitotic Effects of Annona Muricata Extracts to Allium Cepa Root Meristems

[1]Therese Anne Limbana, [1]Louis Concepcion, [1]Azel Baron, [1]Blessy Gascon, [1]Giselle Godin, [1]Mary Margaret Amistoso.

[1]West Visayas State University College of Medicine, Philippines

Abstract

Guyabano (*Annona muricata*) harbors a wide array of bioactive compounds that confer significant therapeutic properties. In an attempt to uncover its anti-cancer properties, we investigated the antimitotic effects of guyabano seed extracts on onion (*Allium cepa*) root meristems.

Methods

The present study utilized the Posttest Only Control Group Design. Onion root meristems were exposed to four distinct concentrations of aqueous and ethanolic extracts of guyabano seeds for 48 hours. For baseline conditions, the negative control group was treated with water whereas the positive control group received methotrexate.

Results

Among the various concentrations of guyabano seed extract treatments, the 800mg/100mL concentration exhibited the lowest mean mitotic index (MMI) in both aqueous and ethanolic solutions (40.33±24.50 and 32.00±9.85, respectively). However, statistical analysis revealed no significant differences between the aqueous and ethanolic treatment concentrations when compared to the negative and positive controls (P=0.40).

Conclusion

The antimitotic effects of aqueous and ethanolic seed extracts of guyabano on onion root meristems were found to be insignificant. However, the lower MMI of both aqueous and ethanolic concentrations of 800mg/100 mL guyabano compared to positive control (methotrexate) warrants further research to assess the antimitotic effects of guyabano.

Keywords: Meristem; Anticancer; Antimitotic; Bioactive Compounds; Mitotic indices.

Article Information:

Article history: *Received:* 01/03/2023 *Accepted:* 10/03/2023 *Online:* 16/03/2023 *Published*: 16/03/2023

DOI: https://doi.org/10.60087/jklst.vol2.n1.p180

Introduction

Annona muricata, also known in other Western countries as soursop or graviola, and locally as guyabano, is a small evergreen tree that is usually 5 to 7 meters high, with large, glossy, dark green leaves. The plant is also native to sub-Saharan African countries that lie within the tropics. It belongs to the family Annonaceae and genus Annona. The plant produces a large edible fruit, usually shaped like a heart, with a diameter of 15-23 cm. and can have a mass of up to 2.5 kgs. The fruit has a sour, white pulp and a core of indigestible, black seeds. Guyabano is believed to possess an abundant source of healing properties. It has a long history of use in herbal medicine as well as a lengthy recorded indigenous use. All parts of the plant, including the bark, leaves, fruit, seeds and roots, are used in natural medicine and each part is attributed to different medicinal properties [1]. Traditionally, the bark as well as the leaves and roots are used as antispasmodic, antidiabetic, smooth muscle relaxant and sedative. The crushed seeds are also used to kill parasites and leaf tea is used for liver problems. The fruit and fruit juice are also taken to cool fevers and even increase mother's milk after childbirth [2].

There have been experiments conducted to study the biochemical properties of guyabano and findings show that it contains many bioactive compounds and phytochemicals. Some of the phytochemicals isolated are annonaceous acetogenins, alkaloids, carotenoids, flavonoids and vitamins B and C. In addition, annonaceous acetogenins are only found in the Annonaceae family and these compounds have been documented to possess cytotoxic properties, such as antimicrobial, antiparasitic, pesticidal and even antitumorous [3]. The presence of several bioactive compounds in guyabano has prompted many researchers to investigate its anticancer potential. Scientific studies suggest that various acetogenins are effective in inhibiting the growth of several human cancer cell lines and even those that are multi-drug resistant. Other findings show that these compounds are better than established anticancer drugs when it comes to specific selectivity of target cells. Research is also ongoing to test the potency of acetogenins in suppressing human immunodeficiency virus (HIV) infection [4].

Although there have been experiments conducted in testing the cytotoxic activity of bioactive compounds found in guyabano to human cell lines and experimental animals, it is still beneficial to test its antimitotic activities in plant cells. It is of interest to the researchers to investigate if the seed extracts of guyabano can have the ability to retard or inhibit the growth of plant cells using the *Allium cepa* test. This study has the purpose of supporting other research findings suggesting that guyabano has anticancer potential.

Conceptual Framework

Independent Variables

Dependent Variable

Aqueous and Ethanolic Seed Extracts:

Treatment Concentrations:
100 mg/100mL
200 mg/100mL
400 mg/100mL
800 mg/100mL

Negative Controls:
Distilled Water
70% Ethanol

Positive Control:
Methotrexate

Mean Mitotic Index (MMI)

Methods

Study design

This experimental study utilized a true experimental design, specifically the Posttest Only Control Group Design [5] in determining the antimitotic effect of aqueous and ethanolic guyabano seed extract on onion root meristems.

Study Setting

The guyabano fruits were collected from the trees found in Brgy. Calumanggan, Bago City, Negros Occidental and were harvested in the month of May 2012. The collection and preparation of seeds were conducted in Iloilo City. The

onion (*Allium cepa*) samples were purchased from a local market in Iloilo City. The seed extraction and chemicals used were procured from West Visayas State University (WVSU) Central Science Laboratory. The onion root treatment and microscopy were conducted at the WVSU College of Medicine Laboratory.

Study Period

The study was conducted from the months of October 2011 to March 2013 in Iloilo City. The collection of guyabano fruits and onions was done last May 2012. The experiment proper, which included seed preparation and extraction, treatment and exposures, and microscopic analysis, was conducted from May to July 2012. Data processing and analysis were done from July to February 2013.

Study Population

Inclusion Criteria: For the onion bulb selection, each should have a weight ranging from 5-10 grams and should be free from spoilage and dents.

Exclusion Criteria: Onion bulbs having 5 or less roots, 7 or more days old, rotten bulbs, bulbs without or rotten roots, and bulbs with insect or rat bites were excluded from the study.

Study Maneuvers

Collection of Plant Materials: The guyabano (*Annona muricata*) fruits were obtained from Brgy, Calumanggan, Bago City, Negros Occidental. A day or two upon delivery in Iloilo City, the fruits were brought to a biologist/botanist in West Visayas State University for identification and verification. Approximately 1 kilogram of seeds was manually extracted and washed three times with tap water. The seeds were air dried in the shade at room temperature for 72 hours. The dry seeds were pulverized into finely powdered form using mechanical grinder and were packed in a clean, air-tight, water-proof and light-protected polythene container for not more than a week until the moment of use.

For the onion samples that had undergone preparatory rooting, 100 red, common onions (*Allium cepa*) were purchased from a local market in Iloilo City. Upon collection, the onions were kept in a cool, dry area until the time of use.

Preparation of Aqueous and Ethanolic Guyabano Seed Extracts: For seed extraction, the previously packed 1 kilogram of powdered seeds was divided into half. Five hundred grams of seeds were used for aqueous extract and the remaining seeds for ethanolic extract. Both extracts had undergone different procedures. The extraction processes were expected to produce semi-solid crude extracts which were eventually diluted by aqueous and organic solvents.

Preparation of Treatment Concentrations: In this study, the experimental groups were treated to two sets of treatment, namely, the aqueous and ethanolic seed extracts and were exposed for 48 hours. Each treatment had four different

concentrations with corresponding labels. In addition, the control group was exposed to three treatments. For negative controls, distilled water and 70% ethanol were used, while methotrexate [6] was used as positive control. Both the experimental and control groups had 3 replicates [7] for each treatment.

The aqueous extract was prepared with some modifications based on the methodology performed by Saradha et al. [8] as follows: 500 grams of powdered guyabano seeds were boiled in 1 liter of distilled water with occasional stirring for 2 hours. The extract was filtered using Whatman no. 1 filter paper and the filtrate was evaporated and concentrated *in vacuo* under reduced pressure at 60°C for dryness using Rotavapor. The extract yielded was diluted with distilled water based on the different aforementioned concentrations of aqueous extract.

The ethanolic extract was prepared with some modifications based on the methodology performed by Vilar et al. [9] as follows: 500 grams of powdered guyabano seeds were exhaustively extracted with 1 liter of 70% aqueous ethanol at room temperature for three days. Alcoholic solution was filtered using Whatman no.1 filter paper and then concentrated under reduced pressure at 40°C for dryness. The crude ethanolic extract was transferred to glass flasks filled to the top and was kept at 4- 5°C until the moment of use. The crude ethanolic extract of guyabano that was used in the experiment was dissolved in 70% ethanol-water solution based on the different aforementioned concentrations of aqueous solution.

For the preparation of different concentrations of aqueous extract, the 100 mg/100mL concentration was prepared by diluting 100 mg of the crude extract in 100 mL distilled water. The 200 mg/100 mL was prepared by diluting 200 mg of crude extract to the same amount of distilled water. The 400 mg/ 100 mL and 800 mg/100 mL were diluted also with the same amount of distilled water.

For the preparation of different concentrations of ethanolic extract, 100 mg, 200 mg, 400 mg, and 800 mg of the crude ethanolic extracts were individually diluted with 100 mL of 70% ethanol to come up with the 100 mg/100mL, 200 mg/100mL, 400 mg/100mL, and 800 mg/100mL treatment concentrations, respectively.

Preparation of Solutions for Control Group: This experiment used distilled water to act as negative control, as supported by a research review [7]. The amount to be used for the treatment of onion bulbs was the same with the experimental group. For positive control, methotrexate, an anticancer drug, was used in the study. This was adopted from the study conducted by Thenmozhi and Mahadeva Rao [6] which utilized methotrexate as standard control in an *Allium* test. The standard amount of the drug was 0.10 mg/ml, and the same amount was used in this study. The procured vial of methotrexate solution had an amount of 50mg/2mL. To come up with 0.10 mg/mL of the standard

amount of concentration of antimitotic agent, 4 mL of methotrexate was withdrawn from the vial and diluted with 100 mL of distilled water. The use of ethanol as another negative control was supported by Tedesco and Laughinghouse [10] who emphasized that if an extract will be diluted in a product such as ethanol, the same diluting agent should also be included in one of the treatments. One hundred milliliters of 70% ethanol was used in the study as negative control.

Pre-treatment and Exposition Methods Using Allium Test: The *Allium* test used in the study was a combination of procedures based on different research reviews [6,7,10]. For the sampling procedure, the study utilized the Simple Random Sampling [5] through drawing of lots in assigning each onion to the different concentrations and control. A coding system was used to label the different treatment concentrations, control and samples. Initially, all sample onion bulbs were carefully scraped at the brownish bottom plate without removing the root primordia to promote the emergence of new growth. The outer scales of the bulb were removed. To set up the experiment, the onions were placed initially in small 100 mL plastic cups containing tap water (pH=5.5-8) for approximately 6 days at room temperature allowing the rootlets to grow. The base of the bulb should reach the medium surface. The roots were protected from direct sunlight to minimize fluctuation of the rate of cell division. The onion samples were stored in a dry, cool environment until proper rooting was attained.

After 6 days of rooting, thirty three onion samples that had rooted adequately were chosen among the 100 pre-grown onions and were used as sample populations. The individual onions were transferred to another set of plastic cups containing the control and treatment concentrations. The experimental group was treated to two sets of treatment: aqueous and ethanolic seed extract. Each treatment had 4 different concentrations: 100 mg/100mL, 200 mg/100mL, 400 mg/100mL, and 800 mg/100mL guyabano extract. For each sample concentration and control, three pre-grown onion bulbs were used. The new sets of 150 mL plastic cups were filled with at least 50mL of sample and control mediums. The onions were stored in the dark for 48 hours at room temperature.

Maceration of Root Meristems and Preparation for Microscopy: When it comes to the number of rootlets to be cut per bulb, Tedesco and Laughinghouse [10] mentioned that it has already been proven that one rootlet per bulb is enough for observing the damage caused to the DNA of *Allium cepa*, observing the cells after the treatment with mutagenic agents. Our study used one rootlet per bulb, having a total of 3 rootlets per sample concentration and control. The root tips were cut at a length of approximately 10 mm starting from the distal end part and were placed separately into 5 mL pre-labeled test tubes with 2 mL acetic/HCl solution. The root tips were heated for 15 minutes at 50°C and were rinsed in distilled water. Subsequently, the roots were soaked in 1-2 mL of acetocarmine solution. It was removed

after 10 minutes and soaked again in acetocarmine solution. It was repeated three times until the root turned into reddish color.

Thereafter, the root tips were placed on a glass slide and the terminal tips (1-2 mm) were cut off. The rest of the root material and liquid were removed from the slide. A cover slip was placed on the root cells. After that, the cells were squashed by placing layers of filter paper on the cover glass and placed slightly down with the thumb. A cover slip was fixed to the slide with clear nail varnish. Microscopy was followed immediately or the slides would be kept in the freezer up to 2 months to preserve freshness and viability.

Data Collection Methods and Tools: The prepared slides were randomly assigned to serial numbers by a non-group member. Each slide was examined under light microscope at high-power objective in a single blind procedure. The assigned examiners from the research team counted 100 cells for each field for a total of 300 cells per treatment. The decision to consider counting only 100 cells per slide was supported by the study of Ping et al. [11], who believed that at least 100 cells be analyzed per bulb, totalling 300 cells per treatment in case of optimizing time. From the microscopic observation, the number of mitotic figures which included interphase, prophase, metaphase, anaphase and telophase of cell division were recorded. The mitotic index was calculated using the formula,

$$\text{Mitotic Index} = \frac{\text{total number of dividing cells}}{100}$$

The mitotic index was expressed in percentage. The mean mitotic index of all 3 replicates of onion samples in the group was calculated. The root length of each onion sample was measured and included in the analysis of growth inhibition of onion roots to different treatments.

Control of Confounding Variables: Simple random sampling was used to assign the rooted onion bulbs to different treatment concentrations and control in order to prevent sample bias. During microscopy, the prepared slides were randomly assigned to serial numbers by a non-group member. The single blind procedure was employed during the examination of the slides. To prevent the deterioration of microscopic figures, the stained slides were immediately examined for microscopy, if possible. In the event that some of the slides would not be able to undergo examination immediately, the unexamined slides would be kept in the freezer for not more than 2 months, as mentioned by a research review.

Comparative Groups

There were two comparative groups in this study, namely the experimental group and the control group. The experimental group was subdivided into aqueous extract and ethanolic extract. Each extract was further divided into four different concentrations: 100 mg/100mL, 200 mg/100mL, 400 mg/100mL and 800 mg/100mL aqueous and ethanolic extract concentrations. For the control group, it was divided into negative and positive controls. Distilled water and 70% ethanol were used for negative controls, while methotrexate was used for positive control. Thirty three onions were exposed randomly in the assigned experimental and control group mediums. Three onions were assigned for each group of extract treatment concentrations and controls. After the exposure to the following treatments for 48 hours, the sample onions were used in microscopy.

Data Processing and Analysis

The data gathered on the mitotic indices of each onion from the different treatment concentrations and control was recorded. The mitotic indices of three onion samples were recorded and then averaged. The mean mitotic indices of the experimental group were compared to each other and to the control group. The data was entered in Microsoft Excel and was subjected to statistical analysis using One-Way Analysis of Variance (ANOVA) in comparing the mitotic indices among the treatment concentrations and control. If post-hoc analysis was applicable, Duncan's Multi-Range Test and Dunnett's Test were used. Statistical analysis was performed using SPSS v.20.0 software. Statistical significance was determined by a ρ-value of <0.05.

Results

The *Allium cepa* root meristems were treated for 48 hours with the following aqueous and ethanolic concentrations of guyabano seed extract: 100mg/100mL, 200mg/100mL, 400mg/100mL, and 800mg/100mL, along with distilled water and 70% ethanol for negative controls, and methotrexate 0.10 mg/mL for positive control. All the comparisons were made using One-Way Analysis of Variance, given a p-value of < 0.05.

As shown in the table below, the lowest mean mitotic index in the different aqueous concentration was seen in the 800mg/100mL concentration. However, there was no significant difference in the mean. Among the different ethanolic concentrations, the lowest mean mitotic index was found in 800mg/100mL concentration, although no significant differences were observed among the means. The control groups were also compared yet they also did not differ significantly. When the MMI of the lowest aqueous treatment concentration (800mg/800mL) was compared with the controls, the MMI did not differ significantly. The ethanolic extract concentration with the least MMI (800mg/100mL) was compared to negative and positive controls, but showed no significance.

When the lowest aqueous treatment concentration (800mg/100mL) and ethanolic treatment concentration (800mg/100mL) was compared with the negative and positive controls, the mean mitotic indices did not differ significantly. For the comparison of all the aqueous and ethanolic treatment concentrations with the negative and positive controls, their mean mitotic indices still showed no significant statistical differences, given a p-value of 0.40.

Table 1. Comparison of MMI Among Aqueous and Ethanolic Treatment Concentrations and the Control Groups

Treatments and Controls	Mean	SD	p-value
Aqueous Treatment			
Aqueous (100mg/100mL)	58.33	20.79	
Aqueous (200mg/100mL)	51.67	11.06	
Aqueous (400mg/100mL)	57.33	4.93	
Aqueous (800mg/100mL)	40.33	24.50	
Ethanolic Treatment			
Ethanolic (100mg/100mL)	51.00	31.00	
Ethanolic (200mg/100mL)	35.33	20.79	
Ethanolic (400mg/100mL)	38.33	13.43	0.40
Ethanolic (800mg/100mL)	32.00	9.85	
Negative Control			
Distilled Water	71.00	12.49	
70% Ethanol	37.67	27.30	
Positive Control			
Methotrexate	59.00	28.69	

Discussion

The National Cancer Institute defines cancer as a term for diseases in which abnormal cells divide without control and can invade nearby tissues [16]. It arises through a series of somatic alterations in DNA that results in unrestrained cellular proliferation [17].The treatment of cancer includes chemotherapy, surgery, radiation, hormones and immunotherapy [18]. Because of the staggering costs [18], high death rate and serious side effects of chemotherapy and radiation therapy on cancer, many cancer patients seek alternative and/or complementary methods of treatment. Recent researches revolve around the urgency to evolve suitable chemotherapy consistent with new discoveries in cell biology for the treatment of cancer with no toxic effect [19,20]. In this instance, cancer researchers have been mulling over the pharmaceutical potentials and anticancer properties of herbal plants.

Natural products cause the induction of cellular defense detoxification and antioxidant enzymes which can protect against cellular damage caused by environmental carcinogens or endogenously generated reactive oxygen species. These agents are also known to act against cancer cells by stimulating the natural immune defense present in the body. There is also another exciting role played by these compounds in combination with standard chemotherapeutic agents. They can act as an adjuvant by lowering the toxicity and enhancing efficacy of standard drugs used in the treatment of more advanced cancers [21,22].

Many phytochemicals and bioactive compounds have been found in *A. muricata* as scientists have been studying its properties since the 1940's. Its many uses in natural medicine have been validated by scientific research [23]. According to Cordell, [24] another new research strategy for the study of the biological activity of natural products is the search of new biological activities of natural products that have just been studied.

Annonaceous acetogenins are a large class of unique structurally homogenous polyketide (C32 or 34 fatty acid) compounds found in the *Annonaceae* family, which includes both genera *Annona* and *Asimina*. These compounds are potent inhibitors of mitochondrial (complex 1) as well as cytoplasmic (anaerobic) production of adenosine triphosphate (ATP) and related nucleotides. Acetogenin compounds are powerful cytotoxins and display in vivo antitumor, pesticidal, antimalarial, antihelminthic, piscicidal, antiviral, and antimicrobial properties, suggesting many potential useful applications [25]. More than 300 acetogenins have been isolated and fully characterized from either bark, leaves, and seeds of different *Annonaceae* species [26].

The main objective of anti-carcinogenic chemotherapy is to stop uncontrolled cellular proliferation. This has prompted many researchers to conduct systematic surveys of new effective inhibitors with ability to react with cytoskeletal

components and arrest living, dividing cells. Even for traditional herbs-consuming populations, encouraging the use of species with chemopreventive actions could be helpful as part of life expectancy improvement strategies. Herbal products have significantly lower costs, exhibit little or no toxicity during long-term oral administration and are relatively available at large scale [27].

For reasons and justifications stated and cited above, this study aimed to find evidence of antimitotic and possibly anti-carcinogenic activity of the guyabano (*Annona muricata*) seed extracts. This study determined the effect of different treatment concentrations of guyabano (*Annona muricata*) seed extracts on the mean mitotic indices of onion (*Allium cepa*) root meristems and the concentration that best retard the growth of onion cells. The treatment concentrations' antimitotic activities were determined by comparing their mean mitotic indices against the positive (methotrexate) and negative controls (distilled water and 70% ethanol).

The mean mitotic indices did not significantly vary among treatment groups and controls. The mean mitotic index was lowest in the *Annona muricata* 800mg/100mL ethanolic seed extract concentration and was highest in the 100mg/100mL aqueous seed extract concentration among all the treatments, indicating the greater antimitotic potential of the ethanolic extract over the aqueous extract. The ethanolic treatment with the lowest mean mitotic index (800mg/100mL) did not statistically differ with those of the negative and positive controls. All the aqueous and ethanolic treatments' mean mitotic indices did not statistically differ with those of the positive and negative controls. Among all the treatment concentrations, the 800mg/100mL ethanolic extract showed the greatest antimitotic activity potential because it yielded an even lower value than the positive control (methotrexate) despite the fact that the value obtained did not statistically differ from those of the controls. The antimitotic activity of the *A. muricata* has been indicated by multiple studies by revealing the presence of annonaceous acetogenins compounds found in the leaves, seeds and stem which are indeed cytotoxic against various cancer cells [28].

Although methotrexate is an established standard control in the *Allium* test as utilized in a study conducted by Thenmozhi and Mahadeva Rao [6], our study revealed no significant difference between the positive (methotrexate) and negative controls (distilled water and 70% ethanol) which would have afforded more reliable statistical data to support the rejection of the null hypothesis. The insignificant statistical results can be due to faulty performance of the experiment or error in data collection procedures specifically with microscopy.

The inconclusive results of the study can be attributed to several factors. Firstly, the environment to which the onion root meristems were exposed was not strictly controlled. Exposure to light may have significant effect on the mitotic

activity of the root meristems considering that the containers of the different treatment concentrations were not prohibitive of the possibility of allowing light to filter through and affect the growth of the roots. In a study by Matias and Fontanilla [29], they have found that light indeed affects the periodicity and rhythmicity of the mitotic division of *A.cepa* var. *aggregatum* root tips, an alternative to the common onion (*A. cepa).* The mitotic indices for those exposed and unexposed to light were observed to peak between 11 a.m. and 12 p.m., whereas that of light-dark set-up was rhythmic, having an hourly fluctuation, but also showed maximum mitosis between 11 a.m. and 12 p.m. Their study therefore recommended that *A.cepa* var. *aggregatum* root tips be excised between 11 a.m. and 12 p.m. for the *Allium* test. They have also related that the pattern of mitotic activity was not significantly affected by light or the absence of it.

Their findings can somehow explain the wide variation in the mitotic indices of the onion root meristems exposed to the same concentration and control. The presence of light could have somehow triggered the periodic and rhythmic mitotic activity resulting to the great disparity among the mitotic indices considering that some of the concentrations and controls which were situated closer to the glass opening of the incubator may have been more exposed to light than those located far back. The same study also mentioned the possibility of the effect of temperature to mitotic activity but data regarding it was not obtained. Secondly, the timing of the cutting of the roots, ideally between 11 a.m. and 12 p.m. as indicated in the aforementioned study cited earlier, was not strictly observed in our study.

Lastly, an important point to be emphasized in an experimental study is the significance of inter-observer variability specifically with regards to the microscopy of the prepared onion root tip slides. This could also account for the wide variations in the mitotic indices of the onion root meristems exposed to the same concentration and control further resulting to wide standard deviations in the statistical data collected and interpreted.

The study has failed to present statistically significant evidence of the antimitotic activity of the guyabano (*Annona muricata*) seed extracts. But it is significant to note that the 800mg/100mL ethanolic extract yielded an even lower value than the positive control (methotrexate), which renders the recommendation for further and more thorough study on the guyabano (*Annona muricata*) seed extracts as an antimitotic agent justifiable.

Conclusion

Both aqueous and ethanolic seed extracts of *Annona muricata* did not demonstrate significant antimitotic effect when applied in a simple mutagenicity test like *Allium cepa* Test. Although it was shown that the lowest mean mitotic index

was found in 800mg/mL concentration of both experimental groups, there was still poor evidence that can conclude its antimitotic activity when compared to a standard antimitotic substance. Therefore, it is necessary to further investigate the antimitotic properties of guyabano.

Recommendations

To bring further confidence to the results, future researchers may increase the number of samples in each set-up. The total number of cells counted during microscopic evaluation of the *A. cepa* root meristem may also be increased. Higher concentrations of *A. muricata* seed extract can also be used in the experimental set-ups, as it may exhibit anti-mitotic properties at higher concentrations. Different methods of extraction may also be utilized to obtain other substances from *A. muricata* seed that may possibly have anti-mitotic properties. Immediate microscopic analysis upon completion of cell staining may prevent possible deterioration of cells that may produce less reliable specimen for cell counting. Consider utilization of other staining methods including chemicals and techniques to be used to further enhance the cellular image essential in cell counting. Inclusions of different durations of exposure to treatments are also recommended to identify possible time-dependent antimitotic activity of onion cells. Encourage other interested researchers to conduct further studies on the present findings to validate the results of this study and consider evaluating chromosomal aberrations and other genotoxic effects of guyabano extract.

Acknowledgement

The researchers would like to extend their deepest gratitude to the following individuals who contributed significantly for the fulfilment of this study:

To Dr. Edna A. Medez, for her unconditional guidance, bright ideas and invaluable support in the conduct of this study;

To Prof. Charmaine S. Malata, for her excellent suggestions and unquestionable expertise in the field of research;

To Prof. Alexander Balsomo, for his exceptional knowledge and skills in statistical analysis and interpretation;

To Dr. Melanie Jane A. Tendencia, head of WVSU-COM Pathology Laboratory, for lending us with full support the college laboratory for our sampling procedures, slide preparation and microscopy;

To Dr. Gerard L. Penecilla and Ma'am Lisette Cuello of WVSU Biology Department and Central Laboratory , for patiently guiding and allowing us to conduct our experiment, particularly the seed extraction and storage;

To Gascon and Reyes families, for the transport and storage of guyabano fruits, as well as providing us with working area for seed collection and preparation;

And lastly, to God Almighty, for sustaining us with strength and hopes in all of our endeavors.

References

1. Adewole SO, Caxton-Martins EA: Morphological changes and hypoglycemic effects of Annona muricata linn.(annonaceae) leaf aqueous extract on pancreatic β-cells of streptozotocin-treated diabetic rats. African Journal of Biomedical Research. 2006, 9. DOI:10.4314/ajbr.v9i3.48903

2. Taylor L: Preprinted from Herbal Secrets of the Rainforest. Published and copyrighted by Sage Press; 2002.

3. Madhuri S, Pandey G: Some anticancer medicinal plants of foreign origin. Current science. 2009:779-783. https://www.jstor.org/stable/24104514

4. Oberlies NH, Chang C-j, McLaughlin JL: Structure− Activity relationships of diverse annonaceous acetogenins against multidrug resistant human mammary adenocarcinoma (MCF-7/Adr) Cells. Journal of Medicinal Chemistry. 1997, 40:2102-2106. DOI: 10.1021/jm9700169

5. David FP: Understanding and doing research: A handbook for beginners. Iloilo City: Panorama Printing Inc. 2005.

6. Thenmozhi A, Mahadeva Rao U: Evaluation Of Antimitotic Activity Of Solanum Torvum Using Allium Cepa Root Meristamatic Cells And Anticancer Activity Using MCF-7-Human Mammary Gland Breast Adenocarcinoma Cell Lines. Drug Invention Today. 2011, 3:290-296. https://www.semanticscholar.org/paper/Evaluation-Of-Antimitotic-Activity-Of-Solanum-Using-Thenmozhi-Rao/4cb0ef4ed0daf3195e4d6f2ea213292e219071ef

7. 7. Onion (A. cepa) Genotoxicity Test. Laboratory of Ecotoxicology and LCA. Department of Environmental Chemistry, ICT Prague. https://www.scribd.com/document/125972147/Allium-

Cepa-Test-1

8. Jyothi KS, Rao BSS: In vitro antibacterial activity of Cleome viscosa Linn. 2010. http://www.pharmasm.com/pdf_files/08_saradha.pdf

9. Vilar JB, Ferreira FL, Ferri PH, Guillo LA, Chen Chen L: Assessment of the mutagenic, antimutagenic and cytotoxic activities of ethanolic extract of araticum (Annona crassiflora Mart. 1841) by micronucleus test in mice. Brazilian Journal of Biology. 2008, 68:141-147. DOI: 10.1590/s1519-69842008000100020

10. Tedesco S, Laughinghouse IV H: Bioindicator of genotoxicity: the Allium cepa test. 978-953-51-0120-8. 2012. DOI:10.5772/31371

11. Yuet Ping K, Darah I, Yusuf UK, Yeng C, Sasidharan S: Genotoxicity of Euphorbia hirta: an Allium cepa assay. Molecules. 2012, 17:7782-7791. DOI: 10.3390/molecules17077782

12. Stedman's Medical Dictionary. (2013) Antimitotic. Accessed: May 24, 2023: http://www.drugs.com/dict/antimitotic.html.

13. The American Heritage Science Dictionary. (2005). Root Apical Meristem. Accessed: May 24, 2023: https://www.thefreedictionary.com/Root+Apical+Meristem.

14. Extract. (2010). Accessed: May 24, 2023: http://dictionary.reference.com/browse/extract.

15. Definitions for mitotic. (2012). Accessed: May 24, 2023: http://www.definitions.net/definition/mitotic

16. What is cancer? (2011). Accessed: May 24, 2023: https://www.cancer.gov/about-cancer/understanding/what-is-cancer.

17. Anthony S. Fauci EB, Dennis L. Kasper, Stephen L. Hauser, Dan L. Longo, J. Larry Jameson, Joseph Loscalzo: Harrison's principles of internal medicine. 2015.

18. Global cancer facts and figures, 2nd Edition. Atlanta: American Cancer Society. (2011).

Accessed: May 24, 2023: https://www.cancer.org/research/cancer-facts-statistics/global.html.

19. Somkuwar AP: Studies on anticancer effects of Ocimum sanctum and Withania somnifera on experimentally induced cancer in mice. Jawaharlal Nehru Krishi Viswavidyalaya; Jabalpur; 2003.

20. Akerele O: Medicinal plants and primary health care: an agenda for action. Eastern Mediterranean Region Drugs Digest. 1991, 8:11-25. https://pesquisa.bvsalud.org/portal/resource/pt/emr-415

21. Howells L, Manson M: Prospects for plant-derived chemopreventive agents exhibiting multiple mechanisms of action. Current Medicinal Chemistry-Anti-Cancer Agents. 2005, 5:201-213. DOI: 10.2174/1568011053765921

22. Chen C, Kong A-NT: Dietary cancer-chemopreventive compounds: from signaling and gene expression to pharmacological effects. Trends in pharmacological sciences. 2005, 26:318-326. DOI: 10.1016/j.tips.2005.04.004

23. Weniger B, Rouzier M, Daguilh R, Henrys D, Henrys JH, Anton R: Traditional medicine in the Central Plateau of Haiti. 2. Ethnopharmacologic inventory. Journal of Ethnopharmacology. 1986, 17:13-30. DOI: 10.1016/0378-8741(86)90070-x

24. Cordell GA: Changing strategies in natural products chemistry. Phytochemistry. 1995, 40:1585-1612. DOI: 10.1016/0031-9422(95)00444-C

25. Alali FQ, Liu X-X, McLaughlin JL: Annonaceous acetogenins: recent progress. Journal of Natural products. 1999, 62:504-540. DOI: 10.1021/np980406d

26. Duret P, Hocquemiller R, Gantier J-C, Figadère B: Semisynthesis and cytotoxicity of amino acetogenins and derivatives. Bioorganic & medicinal chemistry. 1999, 7:1821-1826. DOI: 10.1016/s0968-0896(99)00135-2

27. FERRER JP, Stoiber T, PARRA AV, et al.: Search of new antimitotics compounds from the

Cuban flora. Boletin Latinoamericano y del Caribe de Plantas Medicinales y Aromaticas. 2011, 10:75-82. https://www.redalyc.org/pdf/856/85618182009.pdf

28. Chang F-R, Wu Y-C: Novel Cytotoxic Annonaceous Acetogenins from Annona m uricata. Journal of natural products. 2001, 64:925-931. Chang F-R, Wu Y-C: Novel Cytotoxic Annonaceous Acetogenins from Annona m uricata. Journal of natural products. 2001, 64:925-931.

29. Matias AMA, Fontanilla IKC: Optimizing the utility of Allium cepa L. var. aggregatum (sibuyas Tagalog) for the Allium Test by elucidating its mitotic periodicity and rhythmicity under varying light conditions. Science Diliman. 2011, 23. https://www.researchgate.net/profile/Ian-Kendrich-Fontanilla/publication/235333120_Optimizing_the_Utility_of_Allium_cepa_L_var_aggregatum_sibuyas_Tagalog_for_the_Allium_Test_by_Elucidating_its_Mitotic_Periodicity_and_Rhythmicity_Under_Varying_Light_Conditions/links/563e57fd08ae34e98c4d9135/Optimizing-the-Utility-of-Allium-cepa-L-var-aggregatum-sibuyas-Tagalog-for-the-Allium-Test-by-Elucidating-its-Mitotic-Periodicity-and-Rhythmicity-Under-Varying-Light-Conditions.pdf