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Original Article

Antiproliferative activity of *Cresentia cujete* fruit and leaf extracts using yeast (*Saccharomyces cerevisiae*) model and genotoxic effect using *Allium* test

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Abstract

Plant-based medicines and medicinal plants demonstrate benefits on using them as cutting-edge anti-cancer treatments. This study aims to evaluate the antiproliferative and genotoxic activity of the mature mesocarp of *C. cujete* leaf and fruit extracts using yeast assay and *Allium* test. The *C. cujete* fruit extract demonstrated the highest percentage of inhibition, lowest percentage of viability, least median growth inhibition (GI_{50}), and the highest level of toxicity among the treatments to the yeast assay. Fruit extract has the lowest mitotic index in the *Allium* test, but exhibits similar high chromosomal abnormalities with DMSO. The fruit extract's antiproliferative and genotoxic properties are on par with the anticancer medication methotrexate (MTX) in terms of efficacy. The GI_{50}, percent viability, mitotic index, and percent aberrant chromosomes are all directly correlated with the treatment that was applied. *C. cujete* fruit extract could therefore be a useful source of natural ingredients for the development of anticancer drugs.

Keywords: *Allium test,* antiproliferation, genotoxicity, calabash, yeast

1. Introduction

Every year, the number of cancer-related diseases keeps rising. Additionally, it is projected to keep rising by millions by the year 2030 (Parkin *et al.*, 2001). The majority of the population cannot afford the highly expensive conventional cancer therapies, such as chemotherapy, which involve synthetic anticancer medications. Therefore, it is essential to hunt for alternative methods, such as easily accessible and reasonably priced herbal medicines (Newman *et al.*, 2003). This began with the identification of *Taxus* and *Catharanthus* compounds as promising anticancer medicines, and the subsequent revelation of their anticancer potential (Butler, 2004).

According to Mondal and Mondal (2012), plants are a powerful source of naturally occurring compounds called secondary metabolites that are utilized to treat a variety of illnesses. Finding novel medications to treat cancer is essential (Edelman, 2006). Many healthcare facilities, pharmaceutical corporations, and biotechnological businesses have developed a large number of chemicals in recent years that show promise in treating different forms of cancer. However, because of their expense and ethical issues, only a small number of medications were preselected and clinically assessed by screening. The goal of the screening procedure is to find substances and their derivatives that inhibit the growth of tumors and cancer by meeting the standards needed to identify the substances that will be employed in the subsequent phase of preclinical trials. For the complete screening process to produce the best predictability of pharmacodynamic action, it must be quick, easy, and inexpensive. Therefore, this study was carried out to discover new drugs from the common plants in our area utilizing some easily accessible and far less expensive model organisms.

The calabash tree (*Cresentia cujete* L.) belongs to the Bignoniaceae family and reaches heights of 6 to 12 meters, and it is utilized in conventional medical systems (Figure 1). The enormous, round, green fruits have a diameter

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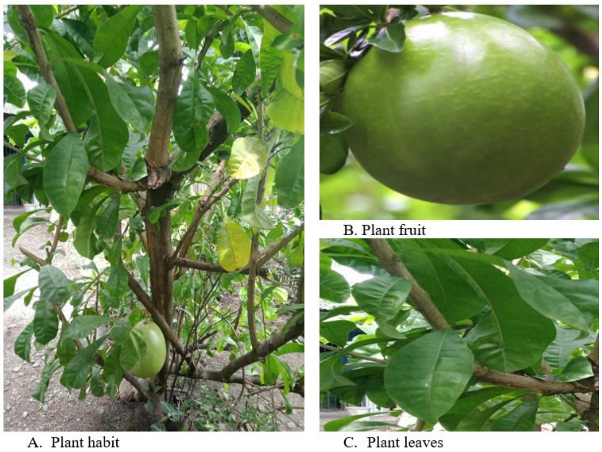

Figure 1. Calabash plant description. A. Plant habit. Calabash tree grows up to 6-12 meters (20-40 feet) tall B. Plant fruit. It has a large, round, gourd-like fruit. The fruit varies in size, reaching up to 30 centimeters (12 inches) in diameter. The outer shell is hard and woody, while the inner pulp is soft and contains seeds. C. Plant leaves. They are simple, alternate, and have an elongated oval shape. They are smooth and glossy, with a dark green color.

of 10 to 30 cm and resemble gourds with spoon-shaped leaves (Arango-Ulloa, Bohorquez, Duque, & Maass, 2009). The fruit and leaf extracts include flavonoids called anthraquinone and quercetin, which have anticarcinogenic properties (Das *et al.*, 2014). The novel aspect of the plant material for its potential anticancer drug fills a vacuum in the literature regarding the use of this material for anticancer screening using the alternative yeast assay for antiproliferation tests of cancer cells and abnormality in chromosomes using the *Allium* test (Latha, Chandralekha, Vilasini, & Panikkar, 1998). Using yeast as a model organism can help shed light on human disease and suggest new avenues for cancer treatment (Kaelin, 2005). Thus, this current study was conducted to assess the antiproliferative and genotoxic activity of *C. cujete* leaf and fruit extracts using yeast assay and *Allium* test, respectively.

2. Materials and Methods

2.1 Plant collection, authentication, and extraction

The mature fresh leaves and fruits were collected in Tigbauan, Iloilo, Philippines (0° 56' 0" N, 122° 27' 0" E). The plant material was collected and authenticated by a plant systematics expert at West Visayas State University (WVSU). The plant materials were cleaned, air-dried, and freeze-dried for 24 hours, then pulverized and stored in airtight containers until use.

2.2 Antiproliferative assay

The yeast *Saccharomyces cerevisiae* BIOTECH 2002 is a pure strain of budding yeast (Jani *et al.*, 2021; Święciło *et al.*, 2024). Research on the budding yeast *Saccharomyces cerevisiae* is the best-studied eukaryotic cycle progression in describing aberrant cell division such as in cancer (Schuyler & Chen, 2021). Pure colonies were isolated by streaking the culture on the YPD plate. Using an inoculating loop, the yeast culture on agar plates was scraped off and aseptically transferred to the nutritional broth solution. Antiproliferative activity was evaluated using the yeast *Saccharomyces cerevisiae* model using the method of Simon (2001) with some modifications. The yeast was inoculated into sterilized potato dextrose broth. It was incubated at 35^0C for 48 hours as the seeded broth. The seeded broth was diluted with sterilized normal saline solution and compared to 0.5 McFarland Standard to get 2.54×10^5 cells (Samantha, Srinivas, & Shyamsundarachary, 2013).

2.3 Preparation of treatments

For the *C. cujete* fruit or leaf extract solution, 4,000 mg of the powdered extract was added with 100 ml distilled water and constantly shaken to make a homogeneous solution. Two (2) milliliters of the solution were added to a 3ml previously prepared broth culture. The first tube was the negative control group without extract but with sterile normal saline solution. In the second and third tubes, 2 ml of extract at 400ug/ml, fruit, or leaf extract concentrations, respectively were added, while in the fourth tube standard anticancer drug methotrexate (MTX) (100 µg/ml) was added. All tubes were incubated at 35°C for 3 hours. All setups were done in three trials and three replicates each, for the validity of the results obtained. After incubation, all the yeast-cultured treatment solutions were centrifuged for 10 mins at 6,000 rpm and then decanted the aqueous solution. The suspended pellet was added with 100 uL phosphate buffer solution (PBS). Two hundred (200) microliters of 0.1% methylene blue was added and incubated again for 5 mins at room temperature. Five (5) microliters of the mixture was added to the hemocytometer and observed under HPO.

2.4 Cell viability count

The methylene blue differential staining method was used to count the number of viable cells. Using a high-power objective, a drop of each treatment solution was applied to a hemocytometer counting chamber. Viable cells were clear, unstained, and oval-shaped. Dead cells, on the other hand, were stained blue and counted in 16 hemocytometer chambers using a manual tally counter. Furthermore, cells were classified as single cells (G1 phase), tiny buds (S phase), medium buds (G2 phase), and large buds (M phase) based on how they budded (Figure 2). The mean quantity of cells was computed. The number of cells per milliliter through percentage of cell viability and percent inhibition of cell viability were calculated using the formulae:

(1) % Cell viability = (Total viable cells/ Total number of cells) X 100;

(2) % Inhibition of cell viability = [(Cell viability of control – cell viability of treated)/cell viability of control] X 100

2.5 Determination of median growth inhibition (GI_{50}) and evaluation

The GI_{50} was computed using the formula:

$$GI_{50} = (T_1 - T_z / C - T_z) \times 100$$

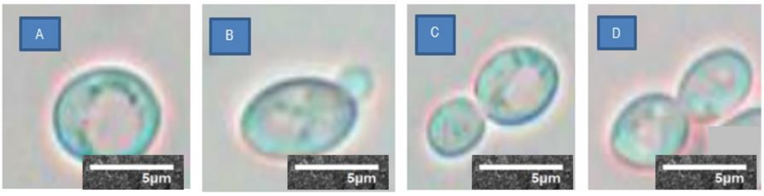

Figure 2. Yeast cells and buds. (A) A single cell (G1 phase) measures about 3-4 micrometers in diameter and is oval or round in shape. (B) Tiny bud (S phase): a small protrusion, or bud that measures 1-2 micrometers, closely attached to the surface of the parent yeast cell. (C) Medium bud (G2 phase): the bud is bigger measures 2-3 micrometers than the tiny bud that starts to grow, and becomes more distinct but remains relatively small compared to the parent cell. (D) Large bud (M phase): the bud is almost the same size as the parent cell measuring 3-4 micrometers.

Where:

T1 = cell viability of negative control

Tz = cell viability of experimental

C = 50

Determination of concentration of the drug GI_{50}. The formula shown below was used:

$$GI_{50} = l_n((T_1 - T_z / T_z) \times 100)$$

If GI_{50} ≤30ug/ml for extract, it has a cytotoxic activity (Alonso *et al.*, 2011).

2.6 Genotoxicity testing

The technique was derived from Fiskesjo's (1988) protocol. In a nutshell, 40±10 g of roughly equal-sized bulbs of onions (*Allium cepa* L.) were purchased from the nearby Iloilo City produce market. The onion bulbs were cultivated for 48 hours at room temperature in the dark with 50 milliliters of tap water until the roots reached around 3 centimeters. The *Allium* test configurations listed below were created in three trials, each with three replicates each consisting of (1) Treatment A: *C. cujete* Fruit Extract, (2) Treatment B: *C. cujete* Leaf Extract, (3) Treatment C: 2% MTX only, and (4) Treatment D: 0.2% DMSO only. The bulbs were immediately put into containers with test samples (one bulb per container). The containers were then closed, well-ventilated, and well-lit, and they were incubated at 22±2°C for 72 hours. After 72 hours, the length of roots that had grown during incubation (newly emerging roots excluded), the number of roots, and the mitotic index were noted. The root tips (2-4 mm) were collected and preserved in a solution of ethanol, acetic acid, and farmer's fluid (3:1). Using a high-power objective bright field compound light microscope, the total meristematic cell count and the number of mitotic cells were manually counted in 5-8 fields of view for each root tip. The mitotic index was calculated by the formula: Mitotic index (%) = Number of dividing cells/ total number of cells X 100.

2.7 Chromosomal observations

The cut onion root tip was immersed in Farmer's fluid. A root tip was put on a slide, macerated for three minutes, and then a drop of 1N HCl was added. Using tissue paper, the extra HCl was absorbed. Safranin was added in little drops and left for five minutes. A coverslip was added after it was passed over the flame three times. The cells were stretched out by gently pressing on them or hitting them with the tip of a pencil. Tissue paper was used to absorb the extra stain. The cells were examined under a high-power objective (HPO) microscope. Records were kept of the many chromosomal abnormalities, including stickiness, laggard, vagrant, and C-mitosis, as well as the phases of mitosis.

2.8 Data collection and analysis

The median growth inhibition (GI_{50}), percentage of viable cells, and percentage of viable cells inhibited by cell inhibition, mitotic index, and chromosomal aberrations of *C. cujete* fruit and leaves extract were evaluated using mean ± standard deviation. The normality test showed a normal distribution of the samples considering it was less than 100 before using the parametric tools. One-Way Analysis of Variance (ANOVA) was used to examine the data, and as a *post hoc* test, the Duncan Multiple Range Test (DMRT) was performed. To evaluate significant correlations between the parameters evaluated, Pearson's r correlation was employed. A statistical difference of $p<0.05$ was deemed significant.

3. Results and Discussion

3.1 Percent inhibition

The fruit extract had the highest percent mean inhibition (36.68 ± 7.31%) of the yeast cells while the leaf extract showed the least percent mean inhibition (5.35 ± 1.97%) (Figure 3). The fruit extract had a higher percent mean inhibitory activity than the positive control, MTX (24.28 ±8.15%) (Figure 3).

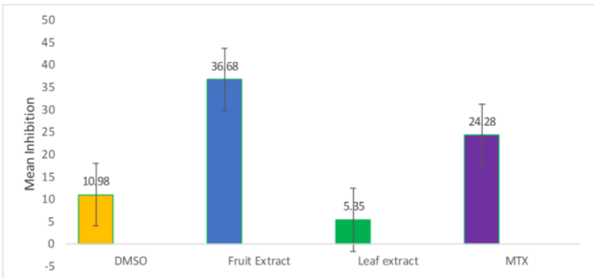

Figure 3. Percent inhibition. Fruit extract showed a significant difference among the treatments indicating the highest inhibitory activity, $p< 0.05$. Each treatment was done in three trials with three replicates each (n=9 per group). *Post hoc*, DMRT indicates differences between treatments, DMSO, leaf extract, fruit extract, and MTX, $p< 0.05$.

3.2 Cell viability

Viable cells were comparatively present in the leaf extract (86.89±4.75%) and DMSO (85.11±11.12%) cases, indicating that these treatments were less effective (Figure 4). The fruit extract (53.88±6.22%) gave the lowest viability of yeast cells indicating that the fruit extract was more effective than the positive control (Figure 4).

3.3 Median growth inhibition

The GI_{50} ≤30ug/ml indicates cytotoxic activity (Alonso *et al.*, 2011). All the values of GI_{50} exceeded the specified value for toxicity level (Figure 5). The fruit extract (55.40 ±6.95%) percent median growth inhibition had the lowest cytotoxicity level revealing that this concentration was cytotoxic to the yeast cell (Figure 5).

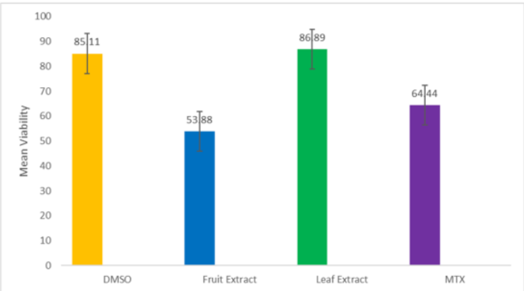

Figure 4. Percent viability. Fruit extract showed a significant difference among the treatments indicating the lowest viability of cells, p< 0.05. Each treatment was done in three trials with three replicates each (n=9 per group). *Post hoc,* DMRT indicates differences between treatments, DMSO, leaf extract, fruit extract, and MTX, p<0.05.

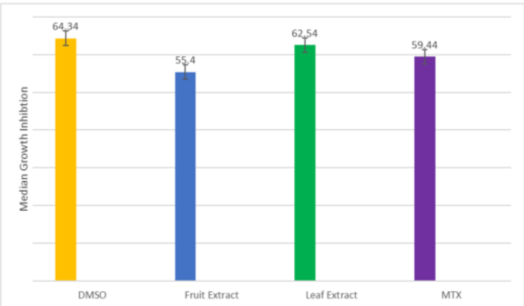

Figure 5. Median Growth Inhibition (GI_{50}). Note: $GI_{50} \leq$ 30ug/ml for extracts is considered having cytotoxic activity. Fruit extract showed a significant difference among the treatments indicating the lowest cytotoxicity level, p< 0.05. Each treatment was done in three trials with three replicates each (n=9 per group). *Post hoc*, DMRT indicates differences between treatments, DMSO, leaf extract, fruit extract, and MTX, p< 0.05.

3.4 Mitotic index

The fruit extract (1.44 ±0.72%) had the lowest mitotic index indicating that the fruit extract suppresses mitosis in the meristematic cells of the onion root tip (Figure 6). The leaf extract (2 ±1.41%) also shows a lower mitotic rate revealing that any part of the *C. cujete* used can slow down mitosis in the onion root tip (Figure 6).

3.5 Aberrant chromosomes

The fruit extract (10.89±5.08%) is comparable to the DMSO (11.22±5.08%) in terms of chromosomal aberrations observed (Figure 7). This may indicate that a lower mitotic index may suppress mitosis by producing more aberrant chromosomes.

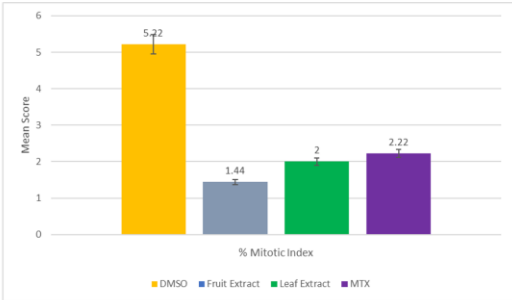

Figure 6. Percent Mitotic Index. Fruit extract showed a significant difference among the treatments indicating the lowest mitotic index, p< 0.05. A lower mitotic rate slows down mitotic activity in the onion root tip. Each treatment was done in three trials with three replicates each (n=9 per group). *Post hoc,* DMRT indicates differences among treatments, leaf extract, fruit extract, and MTX and DMSO only, p< 0.05.

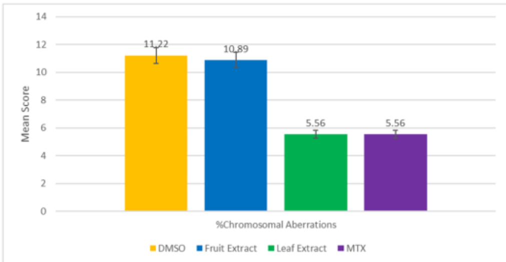

Figure 7. Chromosomal Aberrations. Fruit extract showed a significant difference among the treatments indicating a lower percent chromosomal aberrations to DMSO but higher than the leaf extract and MTX, p< 0.05. More production of aberrant chromosomes may be caused by lower mitotic index. Each treatment was done in three trials with three replicates each (n=9 per group). *Post hoc,* DMRT indicates differences between treatments, DMSO and fruit extract and leaf extract and MTX, p< 0.05.

3.6 Nonviable cell count

The fruit extract showed the highest mean percent inhibition (36.68± 7.31%) (Figure 8). The fruit extract (dead cells, n = 280) gave the highest total nonviable cells killed by the fruit extract used at the G_1 stage containing single-bud yeast cells (Figure 8). The medium bud cells' percent mean inhibition (67.78± 23.24%) at the G_2 and large bud cells (60.12 ± 18.10%) at the M phase were also the target of the fruit extract. Although the leaf extract showed little effect on the inhibition of the yeast cells budding, it appears to have a consistent effect with fruit extract targeting the cells at G_1 (37.67±25.57%) with a single bud (Figure 8).

3.7 Frequency of chromosomal aberrations

The highest chromosomal aberrations were observed in the 0.2% DMSO (f=58) (Figure 9). This was followed by the fruit extract (f=28) while the leaf extract had the least chromosomal aberrations (f=19) (Figure 9). The fruit extract has the lowest mitotic index and with higher chromosomal aberrations observed. The presence of chromosomal aberrations observed in the fruit extract may indicate a way of suppressing the further proliferation of mitosis by shutting down the process through the induction of more aberrant chromosomes. It arrests the cell to enter or further proceed to mitosis specifically during the metaphase stage. It shows that the leaf extract targets more on the production of stickiness chromosomes (f=10). The least chromosomal aberrations produced by the fruit extract were the vagrant chromosomes (f=1).

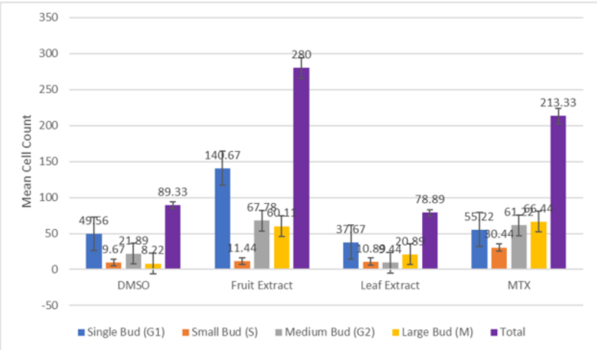

Figure 8. Nonviable Cells Mean Count. The fruit extract showed the highest frequency of dead cells (n=280) at the G1 stage with single-bud yeast cells. This is followed by the medium bud cells at the G2 stage and large bud cells at the M phase which were the target of the fruit extract.

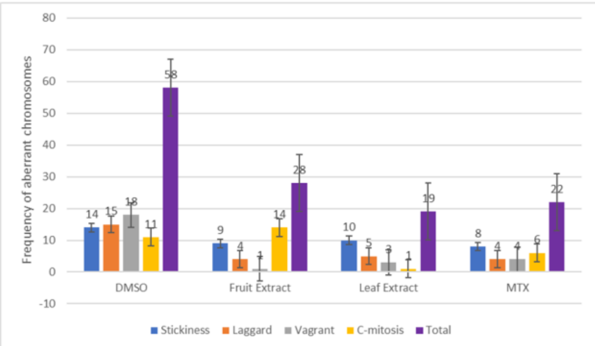

Figure 9. Frequency of the Different Types of Chromosomal Aberrations. The highest chromosomal aberrations were present in DMSO (f=58) followed by the fruit extract (f=28). The chromosomal aberrations observed in the fruit extract compared to the leaf extract may indicate a way of suppressing the further proliferation of mitotic activity.

3.8 Types of aberrant chromosomes

The vagrant chromosome was observed to be dominant in the DMSO group (f=18), C-mitosis in the fruit extract (f=14), stickiness in chromosomes in the leaf extract (f=10), and positive control MTX (f=8) (Figure 10). Chromosome stickiness as illustrated in *Figure 10-A* relates to a sticky surface causing the chromosomes to clump together. This chromosomal aberration is brought about by the strong cytotoxic effect of the maximum concentration of the treatment used leading to the death of the cell. The C-mitotic effect illustrated in *Figure 10-D* is a strong chromosomal aberration like the effect of the mitotic poison colchicine. The effect results in the absence of a spindle and chromosomes arrest in metaphase and appear contracted. Another weak mitotic effect is illustrated in *Figure 10-C* known as vagrant chromosomes wherein the chromosomes do not attach to the spindle apparatus. *Figure 10-B* shows a clastogenic effect – a chromosome-damaging effect. This type of aberration is known as laggard wherein the whole chromosome or its fragments were observed between poles.

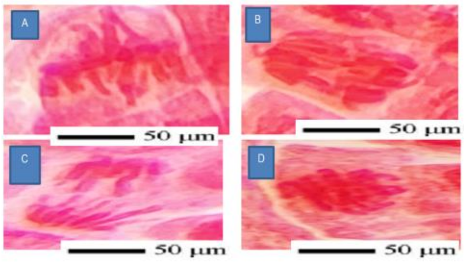

Figure 10. Types of Chromosomal Aberrations. A. Chromosome stickiness results in the clumping of chromosomes together and is present in the leaf extract and MTX. B. Laggard, the chromosomes or their fragments were present at both poles and observed in the DMSO. C. Vagrant, the chromosomes are not attached to the spindle apparatus and are mostly present in DMSO. D. C-mitosis, the chromosomes appear contracted and lack spindle apparatus which are arrested at metaphase and present mostly in the fruit extract.

3.9 Statistical result

The One-Way ANOVA showed significant differences among the treatments used: percent inhibition, F (3,32) =44.591, p (0.000), percent viability, F (3,32) =39.99, p (0.000), GI_{50} F (3,32) =8.013, p (0.000), mitotic index F (3,32) =8.902, p (0.000), and aberrant chromosomes F (3,32) =3.485, p (0.027). The fruit extract showed higher antiproliferative effects in terms of inhibition, viability, and cytotoxicity on the yeast cells. Based on genotoxic effects, the fruit extract showed a significant mitotic index with the lowest mitotic rate but with a significantly higher percentage of chromosomal aberrations. The resulting chromosomal aberrations in the fruit extract indicate a suppression of the further proliferation of mitosis for abnormal cells.

Pearson *r* correlation shows that the percent viability directly correlates with GI_{50} (*r*=0.648, p=0.000) (Table 1). The mitotic index directly correlates with percent viability (*r*=0.409, p=0.013) (Table 1). Aberrant chromosomes directly correlate with the mitotic index (*r*=0.742, p=0.000) (Table 1). A direct positive correlation indicates a positive effect of the treatment used such as in the case of GI_{50} and percent viability; mitotic index and % viability; and % aberrant chromosomes, and mitotic index. However, indirect correlation indicates an opposite effect such as in the case of % inhibition and % viability (less inhibited more viable cells); GI_{50} and % inhibition (higher GI_{50}, less toxic and more

Table 1. Pearson r correlation between parameters assessed. A positive correlation indicates a direct effect of the treatment. These factors directly correlate between (a) mitotic index and % viability, (b) GI_{50} and % viability, and (c) % aberrant chromosomes and % mitotic index. A negative correlation indicates an indirect or opposite effect. These factors indirectly correlate between (a) % viability and % inhibition and (b) % inhibition and % GI_{50}.

Category	1	2	3	4	5
(1) Group					
(2) % Inhibition	r=0.071, p=0.681				
(3) %Viability	r=-0.207, p=0.227	r=-0.903**, p=0.000			
(4) GI_{50}	r=-0.039, p=0.820	r=-0.679**, p=0.000	r=0.648**, p=0.000		
(5) % Mitotic index	r=-0.433**, p=0.008	r=-0.250, p=0.142	r=0.409*, p=0.013	r=0.222, p=0.193	
(6) % Aberrant chromosome	r=-0.450**, p=0.006	r=0.236, p=0.166	r=-0.075, p=0.666	r=-0.207, p=0.225	r=0.742**, p=0.000

Note: N=36; **P< 0.01; *P<0.05.

viable); treatments used, and mitotic index (fruit extract less mitotic index); and mitotic index and viability (higher mitotic index, lesser viability due to inhibitory effect of the treatment used).

3.10 Discussion

This study sheds light on the *C. cujete* fruit and leaf extracts' capacity to inhibit *S. cerevisiae*, a cancer model organism, from proliferating; and to cause genotoxicity in *A. cepa* meristematic cells. Studies that use *S. cerevisiae* as a model organism for cancer research may offer a quicker and less expensive way to screen anticancer medications and determine their cytotoxicity. The results of the *Allium* test reflect the genotoxic activities of *C. cujete* on *A. cepa* root tip cells arresting the specific mitotic stage to inhibit the proliferation of abnormal cells through aberrant chromosomes. The *A. cepa* root genotoxic assay's high sensitivity and strong *in vitro* correlation with mammalian test methods make it an effective method for assessing the genotoxic potential of chemicals (El-Shabbaby, Migid, Soliman, & Mashaly, 2003; Ventura, Angelis, & Marin-Morales, 2008). Plants and animals share physical similarities with mammals and other eukaryotes, as well as a similar response to mutagen therapy (Nefic *et al.*, 2013).

The findings show that in the yeast assay and *Allium* test, fruits are more efficient than leaves because fruits may have a variety of medical benefits that are more potent (Karasawa & Mohan, 2018). Numerous bioactive substances, such as phytochemicals, vitamins, minerals, and dietary fibers, are plentiful in fruits and have been shown to have important therapeutic effects for a range of illnesses (Liu, 2004). The study's findings supplemented data on fruit compositions containing effective bioactive compounds.

A study conducted on the anticancer properties of the fungal endophytes from *C. cujete* leaves using HepG2 cancer cell line by "3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide" (MTT) assay (Seetharaman *et al.*, 2015) showed positive antiproliferative result. Fungal endophytes were isolated from the leaves of *C. cujete* L. Four isolates were identified and molecularly characterized, namely *Nigrospora sphaerica*, *Fusarium oxysporum*, *Gibberella moniliformis*, and *Beauveria bassiana*. The extracts of the four fungal isolates showed antioxidant and anticancer activities. Results revealed that the extracts and compounds from the fungal endophytes of *Crescentia cujete* can trigger apoptosis in cancer cells, leading to their death. The extracts have been shown to cause cell cycle arrest, particularly in the G1 phase, which prevents cancer cells from dividing and proliferating.

The results corroborate the study by Mishra *et al.* (2018) stating that human breast cancer cell line MCF-7 when treated with plant extract *Triphala*, and subjected to budding yeasts gel electrophoresis, revealed a pattern of DNA damage (Sandhya *et al.* 2006). Ayurvedic medicines are proven to be a very effective and safe way to fight the war on cancer. It is also suggested that Withaferin A, a prominent component of *Ashwagandha*, helps reduce cancerous cells (Jani *et al.*, 2021). *Ashwagandha* damages the DNA of budding yeast cell lines when incubated for 24h (Jani *et al.*, 2021). There are similarities in the oxidative metabolism of yeast and the metabolic reprogramming of tumor cells (Guaragnella *et al.*, 2014). The use of budding yeast such *S. cerevisiae* has the possibility of heterologous expression of human genes in yeast allowing new insights to be obtained into the function of mammalian oncogenes/oncosuppressors. The glucose-induced repression of oxidative metabolism is regulated by oncogene homologs in yeast, such as *RAS* and Sch9p, the yeast homolog of Akt. Yeast also undergoes an apoptosis-like programmed cell death process sharing several features with mammalian apoptosis describing also the hallmark of cancer.

The fruit extract from *C. cujete* targets the G1 phase, resulting in irreversible cell cycle arrest. The ability of *C. cujete* fruit extract to inhibit the G1 phase of *S. cerevisiae* may indicate that it has anticancer properties that could target the cell cycle of mammalian cancer cells. Furthermore, the C-mitosis chromosome is the product of the metaphase stage of mitosis, which is the target of the genotoxic effects of *C. cujete* fruit extract (Frescura *et al.*, 2013; Khanna & Sharma, 2013). The fruit extract's cytotoxic components may be the cause of the mitotic index (MI) decrease (Sreeranjini & Siril, 2011). The possible cytotoxicity is explained by the decrease in MI, which also shows that the examined extracts had an antiproliferative effect on *A. cepa* root tip meristematic cells in the yeast assay while being inhibitory and mitodepressive in the *Allium* test of *A. cepa* root tip meristematic cells. Fruit extract's decreased mitotic activity may result from inhibiting the cell's ability to initiate mitosis in the G2 phase of the cell cycle or from suppression of DNA synthesis (Sudhakar, Gowda, & Venu, 2001). There is a decreased amount of ATP to support chromosomal movement, spindle elongation, and microtubule dynamics, as well as a compromised nucleoprotein synthesis (Majewska *et al.*, 2003).

The presence of specific phytochemical components in the extracts may account for the genotoxic effects in *A.*

cepa root meristematic cells and the antiproliferative effects in the yeast assay. Certain naturally occurring substances found in *C. cujete*, including tannins, alkaloids, polyphenols, and flavanols have been linked to chromosomal damage that prevents cells from proliferating further (Perez-Carreon *et al.*, 2002). The components causing the observed chromosomal abnormalities in treatments or the direct effects of certain substances found in the fruit extract may be the source of the observed chromosomal aberrations and yeast cell death.

The study's findings suggest that a candidate medication may be able to treat cancer. Herbal therapy has developed into a relatively accessible, non-toxic, and safe source of substances that treat cancer (Ryu *et al.*, 2011; Shebab, Mahdy, Khan & Noureddin, 2011). Plant-derived metabolites can trigger cell death in cancerous cells which play a part in apoptosis induction in cell lines of some types of cancer (Sohi, Mittal, Hundal, & Khanduja, 2003). The fruit-based phenolic components and a built-in antioxidant are essential in halting the development of cancer and malignancy (Taraphdar, Roy, & Bhattacharya (2001). Research on the prevention and treatment of cancer with herbal medicine has demonstrated that some herb components may have an impact on the onset, development, and advancement of the disease (Ruan, Lai, & Zhou, 2006). Therefore, the utilization of this plant, in particular the fruit extract, may result in the identification of novel, locally accessible anticancer medicines.

4. Conclusions

The current investigation confirms the genotoxic and antiproliferative qualities of *C. cujete* fruit extract, which merit more investigation. The fruit's purported medicinal qualities are derived from its genotoxic and antiproliferative characteristics. This study reveals the genotoxicity of *C. cujete* fruit extract on the meristematic cells of the onion root tip and its anticancer effect against *S. cerevisiae*, a cancer model organism. Its anticancer mechanism of action is attributed to loss of cell viability and cell cycle arrest at the G1 phase. Therefore, *C. cujete* fruit extract could be a good source of natural ingredients for developing anticancer medications. Consequently, it is advised to isolate and identify the *C. cujete* active phytochemicals that prevent cancer.

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Author Contributions

Rey Tantiado: Conceptualization, Formal analysis, Investigation, Visualization, Writing – Original draft, Writing – Review and editing

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