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Chromosomal Aberrations Induced by Calamansi (*Citrus microcarpa* Bunge) Leaf Extract in Onion (*Allium cepa* L.) Root Tip Cells

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ABSTRACT
The genotoxic and cytotoxic effects of aqueous leaf extract of calamansi (*Citrus microcarpa* Bunge) on the onion (*Allium cepa* L.) root tip cells were investigated in this study. The onion root tip cells showed decreased mitotic indices when the concentration of the calamansi leaf extracts was increased. Chromosomal aberrations were observed, such as multiple nuclei, strap nucleus, and pyknotic cells in onion root cells treated with varying concentrations. No chromosomal aberration was observed in control (distilled water). These results indicate that intake of medicinal plants with unregulated dosage may cause genetic damage.

KEYWORDS: *Allium cepa*; chromosome aberration; *Citrus microcarpa*; dosage; medicinal plant

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1. Introduction

Humans have utilized medicinal plants to treat and prevent various disorders since time immemorial [1]. However, the use of medicinal plants in the treatment and prevention of diseases does not imply that they are safe for unregulated use. Studies showed that the uncontrolled use of medicinal plants posed toxicity-related issues in the human body and caused adverse reactions, ultimately resulting in death [2-4].

Citrus microcarpa Bunge plant, locally known as calamansi, was one of the oldest plants with medicinal value. The plant is a member of Family Rutaceae and originated in China as a natural hybrid between a sour, loose-skinned mandarin and a kumquat [5]. It is widely grown in the Philippines and other Southeast Asian countries. It grows to a height of 3 to 5 meters and is smooth and slightly spiky. Leaflets are 4 to 8 cm long and elliptic to oblong elliptic. Petioles are around a centimeter long and narrowly wings. When fully ripe, the fruit is yellow, nearly spherical, 2–3.5 cm in diameter, 6- to 7-celled, and thin-skinned [6]. While the fruits are supposed to help with coughs and colds, the leaf extract may help with hypertension [7-8]. Existing literature reported that the leaves of calamansi showed antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococcus mutans*, and

methicillin-resistant *S. aureus* [9-10]. Owing to its medicinal properties, the leaves of calamansi are typically boiled with water, and the prepared extract is taken by the people at an uncontrolled rate, depending on the severity of the ailment.

The unrefined nature of herbal preparations, combined with a perceived lack of specificity or precision in applying the plant in traditional medicine, may lead to overdosing, resulting in an accumulation of essential and non-essential plant ingredients in the human system. The accumulation can become toxic, especially in persons who rely heavily on unrefined herbal medicines, causing profound biochemical and genetic implications. Using the onion (*Allium cepa* L.) root tip cells test, genotoxic and cytotoxic effects of calamansi leaves can be determined. Therefore, this study evaluated the genotoxic and cytotoxic potential of aqueous leaf extract of calamansi on the cells using the onion root tip cells test.

2. Materials and Methods

Fresh leaves of calamansi were collected from Tubod and Ditucalan, Iligan City, Lanao del Norte, Philippines. About 500 g of leaf samples were collected. The leaves were cleaned and laid out on the laboratory table. For five hours, these were air-dried at room temperature. Leaves were then crushed using mortar and pestle. Crushed leaves were squeezed and filtered with cheesecloth. To remove plant debris, the extract was further filtered with filter paper. The resulting crude extract was then measured and mixed with distilled water to achieve the desired concentrations for the onion root tip cells test. The prepared concentrations were as follows: 2.5%, 5%, and 10% aqueous extract and control (distilled water). Each concentration was replicated three times.

Small onion bulbs were purchased from the local market of Iligan City, Lanao del Norte, Philippines and were carefully cleansed. The old roots and loose scales were removed and rinsed with water. The onion bulbs were placed on vials filled with tap water to initiate rooting, with the base of the onion bulbs touching the water's surface. The vials were checked every 1 h and were refilled if necessary. This method was done for 2 days.

After the roots had sprouted, onions were transferred to the vials containing the calamansi leaf aqueous extract except for the control. Roots, measuring about 0.5 cm from root cap, were harvested every 24 h for 3 days. Harvested roots were placed in vials containing fixative solution (3 parts methanol: 1 part glacial acetic acid) for at most 15 min with proper labeling. The root tips were immersed in 1 N HCl for at least 10 min to prepare it for dissection and then placed back into the container with fixative. One root at a time was transferred on a clean glass slide with a drop of fixative solution. Root caps were cut off and disregarded, and the remaining roots were sliced into very thin lengthwise through a razor blade. The cells were then squashed with the blunt side of the probe. Crashed root tips were stained with acetocarmine for about 3 to 5 min to absorb the stain. The slides were warmed by an alcohol lamp but not to the extent of boiling the stain. Coverslips were then placed on the root tips, and the excess stain was blotted with tissue paper. Cells were observed under a compound microscope HPO scale. Observations were recorded, and photographs were taken. The number of aberrant cells as a proportion of total dividing cells for each treatment was used to

quantify the incidence of chromosome aberrations. The mitotic index was determined for each treatment and control by expressing the number of dividing cells as a proportion of total cells counted.

3. Results

Cell division was normal in the control. Chromosome aberrations were observed in the online root tip cells treated with calamansi leaf aqueous extract. The onion root tip cells treated with leaf extract in all concentrations (2.5%, 5%, and 10%) for 24 to 72 hr showed various types of chromosomal aberrations. These aberrations include strap nucleus, multiple nuclei, pyknotic nucleus, sticky (clumping) chromosomes, and dead cells (Figure 1).

The data on mitotic activity and chromosomal abnormalities obtained in onion root tip cells treated with calamansi aqueous leaf extract are shown in Tables 1 and 2. Compared to the control, the mitotic index values in the treated onion root cells were very low, and mitotic index values were observed to drop with increasing concentrations of calamansi aqueous leaf extract. The number of aberrant cells increased when the quantity of calamansi aqueous leaf extract was increased.

4. Discussion

The decreased mitotic index values in the treated onion roots indicate that there are cytotoxic substances present in the calamansi leaf aqueous extract which could have caused the inhibition of mitotic activities [11-13]. On the other hand, no chromosomal aberrations were observed in the control.

Strap nucleus and clumping of chromosome were observed more frequently in 2.5% concentration from 24 to 72 hr treatment of the leaf extract. The activity of alkaloids and phenolic compounds produced in the leaves may cause chromosomal clumping [14]. Alkaloids and phenolic substances inhibit a range of enzyme systems, including those involved in energy production, cell membrane integrity, and structural component creation [15].

Multiple nuclei frequency was found in cells treated with a 5% concentration of calamansi leaf aqueous extract for 24 to 72 hours. Multiple nuclei are commonly caused by acentric fragments or lagging chromosomes that fail to merge into the daughter nuclei during mitotic telophase, resulting in cell death due to primary gene loss [16]. The production of multi-nuclei in onion root meristems or any other cell is a sign of chromosomal breakage and disruption of the mitotic process caused by spindle abnormalities [17]. Multi-nuclei are thought to be a sign of real mutation impact [16]. The induction of multi-nuclei suggests that the tested mixtures contained constituents that are clastogenic or are spindle inhibitor.

Pyknotic and dead cells were observed in cells with 10% concentration of calamansi leaf aqueous extract from 24 to 72 hr of exposure. Pyknosis is the irreversible condensation of chromatin in the nucleus of a cell undergoing necrosis or apoptosis [18]. It may be due to the presence of tannins, a naturally occurring phenol in citrus leaves [14]. Tannins have been recently found to have cytotoxic effects in cells [19]. Studies showed that tannins could decrease the viability of cells and reduce the growth

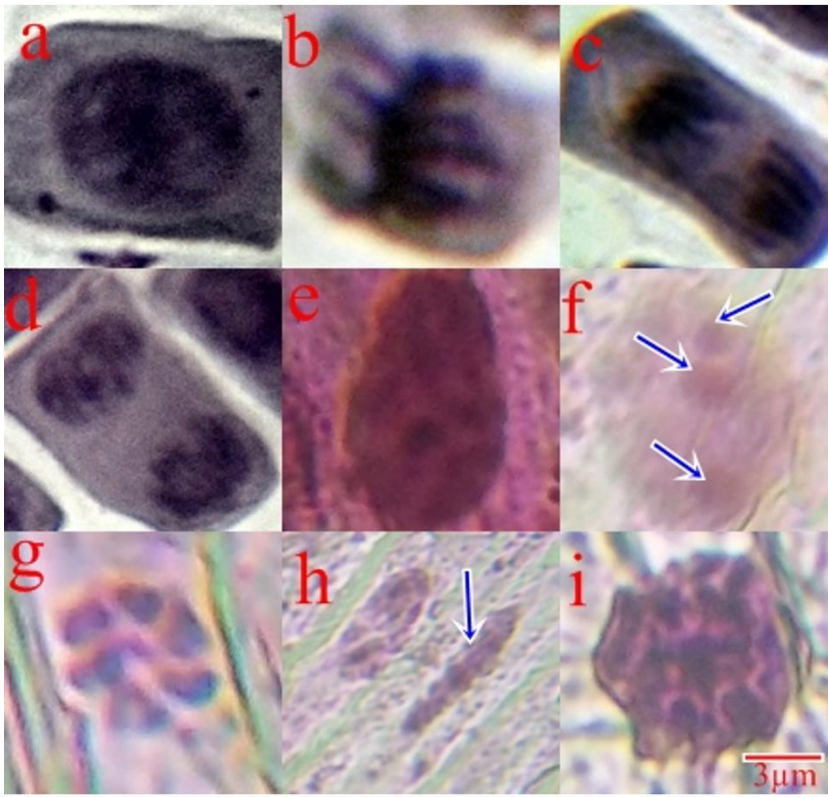

Figure 1. Normal stages of cell division – (a) prophase, (b) metaphase, (c) anaphase, and (d) telophase. Chromosomal aberrations – (e) dead cells, (f) multiple nuclei, (g) pyknotic cell, (h) strap nucleus, and (i) clumping (sticky) chromosome.

rate of animal cells and any other cells [20-21]. These abnormalities show evidence of mutagenic and cytogenic activity of calamansi leaves.

5. Conclusions

The leaves of calamansi may have a healing effect in individuals suffering from hypertension and bacterial infections; however, its unregulated dose is potentially toxic. The actual leaf contents of the plant responsible for the chromosomal aberrations observed were not ascertained in this study. Still, they are taken along with the components acting on the ailments that calamansi is known for. Hence, this study calls for caution in the unguided use and consumption of herbal medicines. Potential cytotoxic and genotoxic substances have an adverse effect on the genetic systems of individuals who practice traditional remedies.

Table 1. Mitotic activities in onion root tip cells treated with calamansi leaf aqueous extract.

Concentration	Prophase cells	Metaphase cells	Anaphase cells	Telophase cells	Total dividing cells	Total counted cells	Mitotic index
Control	123	22	16	8	169	200	84.50
2.50%	12	0	0	0	12	100	12.00
5%	5	0	0	0	5	138	3.62
10%	3	0	0	0	3	128	2.34

Table 2. Chromosomal aberrations in onion root tip cells treated with calamansi leaf aqueous extract.

Concentration	Total counted cells	Total dividing cells	Strap nucleus	Multiple nuclei	Sticky chromosome	Pyknotic nucleus	Dead cells	Total aberrant cells	Aberration incidence
Control	200	169	0	0	0	0	0	0	0
2.50%	100	30	23	0	5	3	4	35	1.17
5%	138	5	0	9	1	0	7	17	3.40
10%	81	3	0	7	0	8	6	21	7.00

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Conflict of Interest Statement

The authors declare no conflict of interest.

Author Contributions: Both authors have contributed equally. They have approved the final version of this manuscript.

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