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Antibacterial Activity and Genotoxicity Assays of Lanzones (*Lansium domesticum)* Seeds Extract

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Lansium domesticum Corr. from Dumaguete City, Negros Oriental, Philippines seed extract were tested for qualitative antibacterial activity against *S. aureus* and *E. coli* and genotoxicity through *Allium cepa* assay. Five concentrations of 1250, 1000, 750, 500 and 250 ug/ml of *L. domesticum* seed extract were subjected to the antibacterial testing; and 100 to 25 percent) for genotoxicity using *Allium cepa* assay. Antibacterial activity was determined qualitatively while genotoxicity was determined by mitotic index and chromosomal aberrations.
Results showed that extract of *Lansium domesticum* seeds antibacterial activity was recorded at 1250 µg/ml, 1000 µg/ml. Genotoxicity test result showed that *L. domesticum* seed extract did not inhibit cell replication but higher concentrations resulted to some chromosomal aberrations.

Keywords: Genotoxicity, *Lansium domesticum, Allium cepa* assay

Introduction

Numerous fruits are medicinally valuable yet many of them remain unexplored. With the growing population and the need for organic medicinal products, fruits can be utilized and investigated for development of potential organic therapeutic product. One of the most commonly available fruits in the Philippines that is widely marketed is lanzones. It is much esteem fruit in the Southeast Asia and is being considered of interest for cultivation in the Western countries (Sayre and Sayre, 2008). Lanzones (*Lansium domesticum*) also known as langsat, is a tree from the Meliaceae family which are widely cultivated mainly for local consumption and is native in the Southeast Asia, and is mostly popular because of its small brown round fruits with sweet flesh and bitter tasting seed (Tilaar *et al.*, 2008).

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Several studies have been conducted for the potential pharmaceutical action of Lanzones. It has been shown that its seed extract has interestingly exhibited a great anti-microbial activity inhibiting growth of Gram-positive bacteria while moderately having a good inhibition on the growth of Gram-negative bacteria (Alimon *et al.,* 2014).In the Philippines, lanzones is used for deworming and ulcer medication by pounding the seeds and mixing it with water. Traditional medicinal use of the seeds also includes eating it directly to cure Malaria (Rubite *et al.,* 2002; Sayre and Sayre 2008). Moreover, the fruit peels are dried and burned to serve as mosquito repellent.

Phytochemical screening of the fruit peel shows high intensity for sugars and alkaloids. Compared to other fruit trees (mangosteen, ponkan, dalandan, pomelo, longgan, and rambutan), it was only lanzones that shows positive results to all tests related to alkaloids (Solidum, 2012). A review conducted by Tilaar *et al.*, 2008 showed that several biological activities of the fruit showed anti-oxidant activity against DPPH free radical and anti tyrosinase activity and it has good cosmetic effect. Dermatologically, the extract is safe to use.

The potential of lanzones seeds for medicinal use is promising however there are minimal investigations into the potential toxicity on their extracts and its antibacterial property. Moreover, the biological activity of Lanzones is still yet to be explored extensively. This study aimed to determine and evaluate the toxicity of Lanzones (*L. domesticum*) seed aqueous extract and its anti-bacterial property.

Materials and methods

Preparation of Aqueous Extract

The seeds of *L. domesticum* from fresh Lanzones fruits were obtained from Dumaguete City, Negros Oriental, Philippines and disinfected with sodium hypochlorite then rinsed with distilled water thrice Cleaned samples were air dried and pulverized mechanically using pulverizing blender and kept in a tightly locked wide mouthed amber bottle.

Fifty (50) grams of the pulverized seeds of *L. domesticum* were heated in water bath in 200 ml of distilled water for 30 minutes. The extract was passed through sterile filter paper disc and stored in sterile amber bottle. Extracts obtained were stored in a tightly stoppered sterile amber bottle (Srisawat *et al*., 2007) and kept refrigerated until use. The weight of leaves before and after boiling and the volume of extract were recorded for the computation of plant extract concentration.

Antibacterial Testing

Two pathogenic bacteria, *Escherichia coli* and *Staphylococcus aureus* were used as test organisms. The bacterial isolates were stored in Nutrient agar slants plus 20% glycerol at -20 C to maintain the two organisms.

Test tubes with Mueller-Hinton broth were inoculated with *E. coli* and *S. aureus* separately and incubated for 4 to 6 hours at ambient room temperature. The growths of the bacteria were adjusted to McFarland No. 0.5 with comparable bacterial density is 1.5×10^8 cells/ml.

The anti bacterial test against *E. coli* and *S. aureus* of different concentrations of *L. domestic* seed extract was determined by dilution broth technique using Mueller-Hinton broth.

Bacterial inoculum of 0.1 ml from the test culture was added to 9.9 ml sterile Mueller-Hinton broth. Each of the five test tubes, was inoculated with 0.1 ml culture and incubated for 24 hours at 26-28 °C. Erythromycin (ERY) for *E. coli* and tetracycline (TET) for *S. aureus* served as positive control and sterile distilled water served as negative control.

Plant Assay Root Propagation

Onion bulbs (*Allium cepa*) were soaked in different concentrations (100%, 75%, 50% and 25%) of aqueous extracts of lanzones seeds with the root section touching the top of the water and incubated three to four days (3 – 4) to induce the growth of the roots from 2-5 cm long, thus the roots were obtained for the experiment.

Genotoxicity Analysis

The use of *A. cepa* as an assay for determining the genotoxic effect of a substance in-vivo has been widely used by researchers over the years because among all assays *A. cepa* L. chromosomal aberration assay have been proven to be effective, sensitive, less costly and used for testing the potential mutagens in both mitotic and meiotic cells (Celik and Aslanturk, 2016).

The root tips were cut and placed on a test tube with 3 ml of fixative solution (glacial acetic acid and ethanol mixture) for 24 hours in order to inhibit the cell division cycle. After submerging in the fixative solution, the roots were washed thrice to 70 % ethanol and soaked for 10 minutes in distilled water.

The meristematic regions of the roots were incised, stained using acetocarmine and mounted on glass slides to spread the cells. The mounted roots were fixed by gently passing over the flame for 30 seconds.

Mitotic index was computed by determining the mitotic cell frequency at the root tip cells divided by the total number of cells observed, as:

$$\text{mitotic index} = \frac{\text{number of dividing cells}}{\text{total number of cells observed}} \text{ x } 100$$

Results

Antibacterial Analysis

Results for antibacterial analysis are shown in Table 1. No bacterial growth of both *E. coli* and *S. aureus* after 24 hours of incubation was observed in T1 (1250 µg/ml) and T2 (1000 µg/ml). Growth of *E. coli* and *S. aureus* were observed in concentration as 750ug/ml and lower concentrations.

Table 3. Growth of *E. coli* and *S. aureus* exposed to different treatments

Concentration (µg/ml)	*Escherichia coli*	*Staphylococcus aureus*
T1 – 1250	-	-
T2 – 1000	-	-
T3 – 750	+	+
T4 – 500	+	+
T5 – 250	+	+
T6 + control	-	-
T7 - control	+	+

+ = has bacterial growth
- = no bacterial growth

Genotoxicity (Allium cepa assay)

Different types of chromosomal aberrations in cells both under mitotic division and resting phase with their frequency and analysis of mitotic index were performed to establish genotoxic effect of *L. domesticum* seed extracts in different concentrations.

Table 2 shows the mitotic index and different types of chromosomal aberrations in root tip cells of *A. cepa* under different concentrations of *L. domesticum* seed extracts. Mitotic index of different concentrations did not show significant variations (ANOVA $P<0.05$). Even when exposed to *L.* domesticum seed extracts, there was no significant change in the mitotic index of cells and there was a decrease percentage in Treatment 5. While Treatment 3 had the highest mitotic index percentage, these results did not demonstrate any

relations on the effect of the different concentrations of extract with with the control which showed that the extract did not inhibit the replication of cells.

L. domesticum seed extract with 100% concentration resulted to high significant effect on the chromosomal aberrations of cells. Most of the aberrations observed were on cells under resting phase with mostly vagrant chromosomes. Moreover, vagrancy of the chromosomes was observed with highest frequency as well as vacuolated and bi-nucleated chromosomes, indicative of the strong genotoxic effect.

The higher the concentration of the extract is, the higher the aberrations were in the cells. However, higher aberrations percentage were observed in 50% compared to 25% because there are more dividing cells than the resting cells observed in 50% compared to other groups, and that some of these cells are then affected by the extract. These aberrations observed in the cells exposed to different treatments may be due to the effect of the phytochemicals present at the *L. domesticum* seed extracts.

Table 2. Mitotic index and different types of chromosomal aberrations in root tip cells of *Allium cepa* under different concentration of *L. domesticum* seed extracts.

Concentrations	Mitotic Index %	average different mitotic chromosome aberrations				average different non-mitotic chromosome aberrations		Total % of Aberrant cells (mean±SD)
		Sticky chromosomes	Bridges	C-mitosis	vagrant-mitotic	Vacuolated / Binucleated chromosomes	Vagrant	
Control (Distilled water)	11.86	0	0	0	0	0	0.33	0.46±0.80
100% extract	12.55	1	7	2	14	19	21	25.34±9.44**
75% extract	11.63	0	0	0	6	1	3	6.34±2.44*
50% extract	15.97	0	0	0	8	1	0	6.62±4.37*
25% extract	9.05	1	1	0	5	0	1	4.01±1.59*

Figure 1 shows aberrations in the chromosomes of *A. cepa* root tips exposed with *L. domesticum* seed extracts. Disrupted chromosomes are labeled with arrows. In c-metaphase, it was observed that chromosomes formed curve shape instead of aligning themselves this could affect the splitting of the chromosomes.

The chromosomes in c-anaphase should move to opposite direction, all chromosomes moved at the same direction and some moved accordingly. Vagrant chromosomes can be seen as threadlike structures without organized

position wherein the threads were not circular and were dispersed. There was also an abnormality in telophase stage where the cell became binucleated; where one had normal chromosomes and the other had vagrant chromosomes.
Bridges aberration can be observed as the threads undergoing anaphase or metaphase that are still attached even when undergoing separation. It was mostly seen as the other chromosomes were moving while some or few of the threads were still attached to each other and failed to separate, vacuolated cells were also observed. In figure 2, the cell labelled *bn* can be observed to have two nuclei with two vacuoles inside.

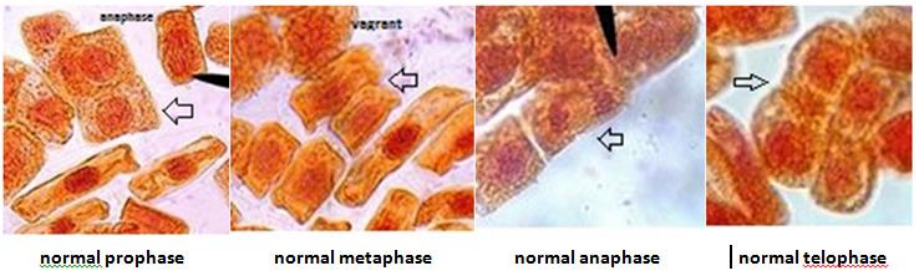

Figure 1. Normal mitotic cell division observed in *A. cepa* root tip cells observed under microscope. *(indicated with arrow)*

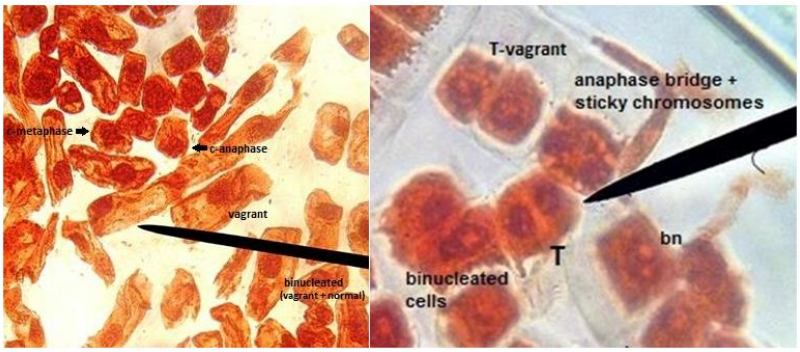

Figure 2. Chromosomal aberrations observed in *A. cepa* root tip cells exposed with *L. domesticum* extract under microscope. (T) normal telophase, (bn) binucleated cells, (T-vagrant) telophase with vagrant chromosomes

Discussion

Results showed that the hot water extract of *L. domesticum* can be used to formulate an antibacterial herbal drug at 1000 µg/ml and can be potentially considered to natural herb against *S. aureus* and *E. coli* infections such as in gastrointestinal infections, urinary tract infections (UTI) and diarrhea. Borges *et al.* (2012) and Borges *et al.* (2014) cited that some phenolic acids (gallic and ferulic acids) and GHP (allylisothiocyanate and 2-phenylethilisotiocyanate) reduced the potential of adhesion to plasmon surface of some pathogenic bacteria, including *E. coli* and *S. aureus*. In other work performed by Luis *et al*. (2014), gallic acid was also able to influence the adhesion properties of methicillin-resistant *S. aureus* (MRSA) to PS. Similar results were obtained by Lemos *et al.* (2014.)

CAs are characterized by changes in either total number of chromosomes or in chromosomal structure which occur as a result of the exposure of chemical treatment. To evaluate the different chromosomal abnormalities, several types of CAs are considered in different stages of cell cycle (prophase, metaphase, anaphase and telophase). CAs are grouped into 2 types, clastogenic and physiological aberrations. Clastogenic aberrations include chromatin bridge/s, chromosomal break/s and ring chromosome/s whereas physiological aberrations include c-mitosis, vagrant/s, stickiness, delayed anaphase and laggard/s.

The term c-mitosis colchicines prevents the assembly of the spindle fibres and results in scattering of the chromosomes over the cells. Normal cycle of mitosis from prophase stage up to telophase stage with the presence of cleavage furrow, chromosomes that are maintained inside the nucleus and normal chromosomes were observed and shown on Figure 1.

Moreover, detection of chromosomal aberration in *A. cepa* roots has been widely used to identify genetoxicity since mitotic index and chromosomal abnormalities are used to evaluate genotoxicity and verifying the mutagenicity of different chemicals.

Conclusion

The aqeous extract of Lanzones seed can inhibit the growth of E. coli and S. aureus at high concentrations. It may not inhibit cell replication but may cause chromosomal aberrations at high concentrations.

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