Lansium domesticum (Lansones) crude seed extract inhibits 4-cell stage mitosis of Tripneustes gratilla embryo and early prophase mitosis of Allium cepa

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Abstract: Mitosis is a hallmark of cancer and plants are potential sources of antimitotic agents. This study investigated the phytochemical contents of Lansium domesticum crude seed extract and its antimitotic activity using Tripneustes gratilla embryo and Allium cepa root tips using concentrations of 0.50%, 1.00% and 1.50% through the time interval between cell divisions, and obtaining the mitotic index in root tips after treatment with the extract respectively. Results of phytochemical screening revealed the presence of triterpenes, steroids and alkaloids. Mitosis of T. gratilla embryo was inhibited at 1.00% concentration up to the 4-cell stage while 0.50% and 1.50% failed to inhibit mitosis. The antimitotic activity of the 1.00% concentration of the crude seed extract was found to be significant until the 4-cell stage when compared to the negative control with p-values less than 0.05. Root lengths of pre- and post-treatment with the crude seed extracts were found insignificant in general except at 1.50% concentration. Mitosis in A. cepa root tips was inhibited until the early prophase on all concentrations tested but reduction of mitotic index was optimal at 0.50% concentration when compared to the mitotic index of the negative control. Results of the study indicate the antimitotic potential of L. domesticum in T. gratilla embryo and A. cepa until the 4-cell stage division at 1.00% concentration and early prophase stage at 0.50% concentration, respectively. Variations in the antimitotic activity of the crude seed extract on the two models of mitosis used in this study can be attributed to the inherent differences in the cell division of T. gratilla and A. cepa.
Keywords: mitosis, Lansium domesticum, Tripneustes gratilla, Allium cepa, mitotic index

INTRODUCTION
An estimated 189 of every 100,000 Filipinos are afflicted with cancer while 4 Filipinos die of cancer every hour or 96 every day (Roxas, 2015). Today, almost all Filipino cancer patients is after medical advice only when symptoms are already appearing worse, on advanced stages. (Ngelangel & Wang, 2001). Cancer occurs when chemicals, viruses and free radicals transform cells to abnormal ones which then proliferate in an uncontrolled manner and become tumor masses (Patil, Narayanan, Eibl & Jolly, 2004).

Mitosis is a diverse mechanism involved in the proliferation of actively dividing cells, with the division of duplicated sets of chromosomes and production of two genetically identical daughter cells as the result (Chan, Koh & Li, 2012). Systemic chemotherapy remains the usual form for treating cancer and agents that interrupt the mitotic spindle assembly, called ‘antimitotics’ are also proven successful (Gascoigne & Taylor, 2009). Since cancer cells are particularly fragile during division in mitosis, this serves as a critical intervention point in chemotherapy.

As a treatment for cancer, antimitotic drugs have been utilized as chemotherapeutic agents which targets microtubules, responsible for the formation of the mitotic spindle, which is important for proper chromosomal separation during cell division (Tysganov et al., 2013). The goal of treatment is to cure the cancer, or lengthen survival in patients while retaining the highest possible quality of life (Siegel et al., 2012).

Roughly more than 60% of the currently utilized anticancer chemotherapeutic medications are inferred in natural sources, such as plants with various phytochemicals which are vital and fundamental anticancer operators (Stankovic et al., 2011). Most of the plant derived anti-cancer drugs affect microtubule dynamics, disrupt some biological processes and modify signaling pathways resulting to apoptotic death (Sehgal, Roy & Kumar, 2006). Lansium domesticicum Corrêa is from the family Meliaceae locally known as ‘Lansones’ (Yapp & Yap, 2003) (Figure 1). Several tetranoterpenoids and triterpene compounds, lansiolides, lansionic acid, etc. were recovered on its seeds (Saewan, Sutherland & Chantrapromma, 2006) with mild toxicity against brine shrimp (Tilaar, Ranti, F.d, Wasitaatmadja & Win, 2008),
insect feeding deterrent activity as well as antimalarial activity (Ragasa, Labrador & Rideout, 2006).

Figure 1. L. domesticum A. fruits B. Seeds

This study has evaluated the phytochemical constituents of L. domesticum seed extracts; its antimitotic activity on T. gratilla embryo and Allium cepa root tips as test system for both animal and plant; determined the effective concentration at which antimitotic activity was mostly evident; and compared the two methods. Since proven effective, L. domesticum seeds can be further studied so that it can be utilized and added to the list of potential plant-based anti-cancer agents.

MATERIALS AND METHODS

Plant and T. gratilla collection and identification

Fruits of L. domesticum (Lansones) were purchased from Cuenca, Batangas and Laguna; T. gratilla (collector urchin) were purchased from Mabini and Malitam, Batangas; and A. cepa (onion) bulbs were purchased from the local market, which were all identified at the National Museum of the Philippines (Appendix A). The seeds of L. domesticum (Lansones) were washed under running tap water and shade dried. These were homogenized into coarse powder and stored in an airtight container until further use (Parekh, Jadejah & Chanda, 2005). T. gratilla were kept in an aquarium with seawater, oxygen as aerator, and sea grasses for food.
Preparation of plant extract
A 350g of the prepared ground plant seed powder was macerated with 1L of methanol in an airtight, clean container for 2 days at room temperature with occasional stirring and shaking, the mixture was filtered with the use of Whatman filter paper and was mixed again with methanol for another 2 days and was filtered again. Using a rotary evaporator, the obtained filtrate was concentrated at 39°C and at a reduce pressure (Apu et al., 2010). The crude extracts were placed in glass beakers and their weights were obtained (Appendix B-I).

Phytochemical Screening
Test for triterpenoids: The extract was dissolved in chloroform and few drops of sulphuric acid were added. The formation of red brown color at the interface showed the presence of triterpenes (Roopalatha & Vijay, 2013).

Test for alkaloids: The extract was dissolved in methanol, 5 ml of Dragendorff reagent was added and the formation of red to orange precipitate indicates the presence of alkaloids (Zeidan, Hijazi, Rammal, Kobaissi & Badran, 2014).

Test for tannins: The extract was dissolved in distilled water, then a few drops of ferric chloride were added. Formation of a dark blue or greenish black color indicates the presence of tannins (Sugumar, Karthikeyan & Gowdhami, 2015).

Test for steroids: The extract was dissolved in chloroform, then few drops of concentrated sulphuric acid were added. Formation of red color in the lower layer indicates the presence of sterols (Rajendra, Magadum, Nadaf, Yashoda & Manjula, 2011).

Preparation of experimental samples
a. T. gratilla assay:
The crude extracts obtained were weighed and dissolved in 20ml of sea water per concentration to prepare 0.50%, 1.0% and 1.50% test solutions. Experimental samples were stored in sterilized vials until use (Appendix B-III).

b. A. cepa assay:
The crude extracts obtained were weighed and dissolved in definite volumes of tap water per concentration to prepare 0.50%, 1.0% and 1.50% test solutions. Experimental samples were stored in sterilized vials and stored at 2-4°C until use.

T. Gratilla Assay
The T. gratilla assay was based on the procedure done by Gutierrez (2016).

**Spawning of T. gratilla:** Identification of the sex of T. gratilla was done through injection of 0.5 ml of 0.5M KCl through the periostral membrane of the T. gratilla. Secretion of orange eggs indicated female T. gratilla while cream-colored semen were indicative of males. Viewing of the secretion under the microscope also validated the sex of the T. gratilla.

For continual shedding and preservation of sperms, spawned male T. gratilla were placed in a container with 30 mL filtered seawater and were kept in a container with ice. The motility of the sperms was checked under the microscope and the secretion which demonstrated high motility were chosen for sperm pooling. Likewise, the spawned female T. gratilla were transferred to a container filled with 30 mL filtered seawater to shed its eggs. The presence of small nucleus in the periphery of the cell membrane and a large amount of cytoplasm as viewed under the microscope indicated mature eggs. Fertilization was done by mixing 12 mL of egg solutions in a sterilized cup with 3 mL of sperm suspension. The eggs were churned and permitted to settle for 15 minutes. Water and other mixtures were discarded then the washed eggs were transferred to 100 ml beaker. The embryos were then divided and placed into three petri dishes for every trial. The embryos were subjected to an ascending concentration from 0.50%, 1.00% and 1.50% of each L. domesticum extract. The test was scaled by determining the time of embryo transformation from one cell to two-cell stage at ten minutes interval.

**T. gratilla Bioassay:** The mitotic assay was patterned on a method done by Gutierrez (2016) with some modifications. 3 mL of sperm suspension was added to 12 ml filtered seawater containing the eggs. The union of sperm and egg was examined under the microscope. The presence of fertilization membrane signaled the onset of fertilization. 5 mL of each egg and sperm suspensions were distributed into the petri dishes corresponding with their assigned treatments. The experimental group received various concentrations (0.50%, 1.00% and 1.50%) of the L. domesticum extract into the 3 petri dishes for every trial. The colchicine drug was used as the positive control group and the filtered seawater for negative control group. Each was carefully viewed to determine the time interval of cell division from the first cell-stage until 8-cell stage (Appendix B-IV).
A. Cepa Assay

The A. cepa assay (Appendix B-V) was based on the procedure described by Ozmen, Basbulbul & Aydin (2007).

Growing A. cepa meristems.

Onion bulbs were carefully unscaled leaving the root primordia in place. (Yuet, Darah, Yusuf, Yeng & Sasidharan, 2012). The bulbs were grown in tap water at room temperature for 3 days.

Exposure to test samples.

When the roots were 2–4 cm in length, the bulbs were treated with the crude extracts at 0.50%, 1.0% and 1.50% concentrations. For the negative control, tap water was used. The treated onion bulbs were incubated at 24 ± 2 °C and the test samples were changed every day. The roots were counted and the root lengths were measured for each onion after 48 hours.

Microscopic studies and determination of mitotic index.

After 48 hours, the roots were settled and were fixed with glacial acetic acid/absolute alcohol in a 1:3 ratio. The root bulbs were kept in the aceto-alcohol solution for 24 h. After fixation, the roots were placed in 70% ethyl-alcohol and kept in the refrigerator. The root tips were gathered and arranged for microscopic studies by standard aceto-orcein squash readiness preparation technique. For the microscopic examination, the root tips were placed into a watch glass to which 9 drops of aceto-orcein and 1 drop of 1 M HCl were added and warmed over a flame of spirit lamp for 2-3 min. These were kept at room temperature for 5 minutes. Afterwards they were submerged in a drop of 45% acetic acid on a slide and squashed under a cover glass. To spread the cells equitably on the surface of the slide, squashing was achieved by applying pressure on the cover glass with the thumb. Mitotic index was reflected in terms of dividing cells/total cells and expressed as percentage (Ozmen et al., 2007).

Statistical analysis

The means of the control and seed extracts/fractions was obtained from descriptive analysis and an Independent-samples test was performed to obtain P values.

RESULTS AND DISCUSSION

Crude L. domesticum seeds extract
In the preparation of crude extract, 21.527 grams of sticky, yellow to dark brown extract (Figure 2) were obtained. The percentage yield was 4.79%.

Figure 2. *L. domesticum* crude seed extract

**Phytochemical screening**

The phytochemical screening (Table 1) of the crude extract of *L. domesticum* seeds used in the study revealed that the crude extract contained triterpenoids, alkaloids, steroids (Figure 3a, 3b, 3d) but negative for tannins (Figure 3c).

<table>
<thead>
<tr>
<th>Phytochemical constituent</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triterpenes</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
</tbody>
</table>

Figure 3. Phytochemical Screening (A) Triterpenoids (B) Alkaloids (C) Tannins (D) Steroids

A positive result in the screening for triterpenes is supported with the study of *L. domesticum* seeds done by Saewan et al. (2006). In his study, five tetranorterpenoid, domesticulide A-E,
were recovered from seed of *Lansium domesticum* Corr. together with 11 known triterpenoids: 6-hydroxymexicanolide, 6-acetoxyxymexicanolide, methyl angolensate, methyl 6-hydroxyangolensate, methyl 6-acetoxyangolensate, azadiradione, lansiolic acid, lansioside B, dukunolide B, dukunolide C, and dukunolide D. On another study done by Tilaar et al. (2008) onoceroid triterpenes, lansiosides and lansic acid were isolated from *L. domesticum* seeds. A positive screening test for alkaloids was correlated with the study of Solidum (2012), which showed the alkaloidal content of *L. domesticum* peels being 0.0312 mg/ml level at 1:9 dilution. A positive result in steroids was supported by the study of Solidum (2012) as well when she found out that fruit peels contain steroids.

**T. Gratilla Assay**

In the investigation of the antimitotic effect of *L. domesticum* crude seed extracts, we have employed the utilization of *T. gratilla* embryo as model for cells undergoing division. Sea urchin embryonic cells has come up as an appropriate model for the investigation of compounds with antimitotic activity mainly because a considerable amount of eggs can be easily gained and fertilized externally (Leite et al., 2012) (Pellerito et al., 2005). This method however was subjected to various limitations that’s why some steps in the procedure were modified. Since simultaneous execution of the whole procedure cannot be done at once, multiple trials of different concentrations were done on different days until the desired result was obtained. With this, the volume used in collecting the eggs and sperm, and the volume used in the fertilization process were also modified to come up with an equal amount (15ml) to be distributed in three petri dishes for further examination of dividing cells. Also, continuous examination of the cells under the microscope was not feasible because they were very prone to drying up, therefore damaging the cells in the process in terms of their morphology and thus, their ability to divide. To come up with a solution, collecting 30ul of the embryo and examination of cells was done every 10 minutes and continued until the 8th cell division was observed.

**Table 2. Mean time interval of the early embryonic developmental stages of T. gratilla eggs treated with the various concentration of L. domesticum extract**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2-cell stage</th>
<th>4-cell stage</th>
<th>8-cell stage</th>
</tr>
</thead>
</table>

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<table>
<thead>
<tr>
<th>Concentration</th>
<th>Negative control</th>
<th>Positive Control (Colchicine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.50%</td>
<td>40</td>
<td>120</td>
</tr>
<tr>
<td>1.00%</td>
<td>41.67</td>
<td>21.67</td>
</tr>
<tr>
<td>1.50%</td>
<td>47.67</td>
<td>20</td>
</tr>
</tbody>
</table>

Shown in Table 2 is the mean time interval of cell division from the onset of the fertilization membrane up to the 2-cell stage, the interval from 2-cell stage up to its division into 4-cell stage, and the interval from the 4-cell stage up to its cleavage into 8-cell stage (Figures 4,A-D). Based from the results, time intervals from the fertilization membrane up to 2-cell were 41.67, 59.67 and 47.67 minutes at 0.50%, 1.00% and 1.50% concentrations respectively, compared to the negative control which was 40 minutes. Mitosis was prolonged most evidently at 1.00% concentration as seen in the time interval of 59.67 minutes at 2-cell stage. The same observation was made at 4-cell stage, with 1.00% concentration still the most effective with 21.67 minutes as result. However during the 8-cell stage, there was a varying effect between the concentrations of the extract. The results were not consistent in prolonging the time interval of division between cell stages because the antimitotic effect of the crude seed extract was not dose dependent but dose specific contrary to the results of the study conducted by Gutierrez (2006). However, the extract did not match the effect of the positive control colchicine, wherein only one cell was able to divide into two-cell stage in a matter of 2 hours, which could possibly be attributed with the compound’s exceptional purity, even after successive dilutions.
Table 3. Comparison of Different Concentration for each cell stage

<table>
<thead>
<tr>
<th></th>
<th>0.50%</th>
<th>1.00%</th>
<th>1.50%</th>
<th>t-value p-value</th>
<th>t-value p-value</th>
<th>t-value p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-cell</td>
<td>1.890</td>
<td>0.132</td>
<td>NS</td>
<td>22.30 0.000**</td>
<td>HS 0.679 0.534</td>
<td>NS</td>
</tr>
<tr>
<td>4-cell</td>
<td>1.890</td>
<td>0.132</td>
<td>NS</td>
<td>4.914 0.008**</td>
<td>S 0.725 0.509</td>
<td>NS</td>
</tr>
<tr>
<td>8-cell</td>
<td>0.728</td>
<td>0.507</td>
<td>NS</td>
<td>0.542 0.616 NS</td>
<td>1.880 0.133 NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Legend: *p-value < 0.05; **p-value < 0.01

Table 3 presents the comparison of the different concentration. Based from the result, there were insignificant differences between the 0.50% and 1.50% concentration with that of all the cell stages observed and on the negative control since all p-values obtained were above the alpha level of 0.05. However, there were significant differences between the negative control and 2-cell (0.000) and 4-cell (0.008) since the obtained p-values were less than alpha level of 0.05. It shows that the antimitotic effect of 1.00% extract on 2-cell and 4-cell compared to the negative control group varies. This delay in the cell division could be due to the combined effects of the phytochemicals present on *L. domesticum* seeds which includes triterpenes, steroids, and alkaloids. As an antimitotic agent, some triterpenes have a potential in targeting microtubules which results in the disruption of microtubule assembly such as lupeol (Saleem, Murtaza, Witkowsky & Kohl, 2009), and ursolic acid (Song et al., 2012). Terpenoids stabilize tubulin polymers thereby subsequently
inhibits the disassembly of microtubules. Steroids act by blocking the G2/M phase of cell cycle, and apoptosis induction. Alkaloids can also bind to tubulin which is a major protein in microtubules, during cell division and inhibiting its formation, thereby inhibiting mitosis (Gutierrez, 2016). However the observed effect cannot be directly established since not all concentrations induced the delay in mitosis. Through the *T. gratilla* assay, the effects of the *L. domesticum* crude seed extract was evaluated and documented.

**A. Cepa Assay**

Antimitotic effect was observed based on the mean root growth before and after treatment with the different concentrations, the cell phases observed on the microscope and calculation of the mitotic index.

![Figure 5. A. cepa roots pre- & post-treatment. A. negative control; B. 0.50%; C. 1.00%; D. 1.50%](image)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Average Root Length pre-treatment</th>
<th>Average Root Length post-treatment</th>
<th>Root length growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>1.88cm</td>
<td>3.26cm</td>
<td>1.38cm</td>
</tr>
<tr>
<td>0.50%</td>
<td>2.2cm</td>
<td>2.5cm</td>
<td>0.3cm</td>
</tr>
<tr>
<td>1.0%</td>
<td>3.06cm</td>
<td>3.31cm</td>
<td>0.25cm</td>
</tr>
<tr>
<td>1.50%</td>
<td>2.95cm</td>
<td>3.25cm</td>
<td>0.30cm</td>
</tr>
</tbody>
</table>

Table 4 shows the average root length of *A. cepa* bulbs which were 1.88cm, 2.2cm, 3.06cm and 2.95cm after exposure on tap water.
initially and before treating with concentrations (Figure 5). There is a specific region in the roots of plants which is the meristematic region where repeated divisions happen (Thenmozhi & Rao, 2011), which was evidenced by root growth. The longer root lengths were used in the higher concentrations because we assumed that the negative control group will still grow in significant lengths. After two days of treating the said roots with the extract, the obtained root lengths were as follows: 3.26cm on the negative control, and 2.5cm, 3.31cm, 3.25cm at 0.50%, 1.00% and 1.50% respectively. Root length growth was calculated by getting the difference between the pre- and post-treatment of the root tips with the extract. Based on the results, the root tip that was continuously exposed with tap water for two days grew 1.38 cm while for the test samples, the root tips exposed to the 0.50%, 1.00% and 1.50% concentration grew 0.3cm, 0.25 and 0.3cm respectively.

Table 5. Comparison of A. cepa (onion) bulbs with the different concentrations of plant extracts

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Mean pre</th>
<th>Mean post</th>
<th>Mean Difference</th>
<th>t-value</th>
<th>p-value</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>1.88</td>
<td>3.26</td>
<td>-1.380</td>
<td>6.900</td>
<td>0.092</td>
<td>Not Significant</td>
</tr>
<tr>
<td>Control</td>
<td>2.20</td>
<td>2.50</td>
<td>-0.300</td>
<td>0.600</td>
<td>0.656</td>
<td>Not Significant</td>
</tr>
<tr>
<td>0.50%</td>
<td>3.06</td>
<td>3.31</td>
<td>-0.250</td>
<td>1.250</td>
<td>0.430</td>
<td>Not Significant</td>
</tr>
<tr>
<td>1.00%</td>
<td>2.095</td>
<td>3.25</td>
<td>-0.295</td>
<td>59.00</td>
<td>0.011</td>
<td>Significant</td>
</tr>
<tr>
<td>1.50%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5 shows the comparison of the negative control and the different concentrations of the extracts. The results between the pre- and post-treatment of the negative control, 0.50% and 1.00% concentrations were found to be not significant since the obtained p-values were greater than 0.01. At 1.50% concentration, a p-value of 0.011 was obtained indicating a significant difference. This means that growth of the negative, 0.50% and 1.00% concentration was the same during pre- and post-treatment while growth at 1.50% differed during pre- and post-treatment. This implied that growth was mostly evident at 1.50% concentration because mitosis took place, which can be correlated with its mitotic index result of 83.73%, higher than the negative control, 0.50% and 1.00% concentrations. This can be supported by the study of Chan et al. (2012) about mitosis which is a diverse mechanism involved in the proliferation of actively
dividing cells with the division of duplicated sets of chromosomes and production of two genetically identical daughter cells as the result.

![Image of dividing cells]

**Figure 6.** Cell phases on various treatments A. Negative control; B. 0.50%; C. 1.0%; D. 1.50

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Interphase</th>
<th>Prophase (Early)</th>
<th>Metaphase</th>
<th>Anaphase</th>
<th>Telophase</th>
<th>Total No. of Cells</th>
<th>Mitotic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control 0.50%</td>
<td>63</td>
<td>241</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>309</td>
<td>79.61%</td>
</tr>
<tr>
<td>1.00%</td>
<td>126</td>
<td>65</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>191</td>
<td>34.03%</td>
</tr>
<tr>
<td>1.50%</td>
<td>148</td>
<td>308</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>456</td>
<td>67.54%</td>
</tr>
<tr>
<td>1.50%</td>
<td>48</td>
<td>247</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>295</td>
<td>83.73%</td>
</tr>
</tbody>
</table>

Table 6 elaborates the number of each cell phases observed on each tested concentrations and negative control. As supported by microscopic examinations of the root tips (Figure 6), cell phases were observed. Out of 309 cells counted on the negative control on one high power field, 241 were in the early prophase, 2 in metaphase, 1 in anaphase, 2 in telophase and 63 in the interphase where cells did not divide. Out of the 191 cells counted at 0.50% concentration, only 65 cells were in the interphase while 126 cells were in the early prophase. Out of the 456 cells counted at 1.00% concentration, 308 cells were in the early prophase while only 148 cells were in the interphase. Out of the 295 cells counted at 1.50% concentration, 247 cells were in the early prophase while only 48 cells were in the interphase stage. No other further cell phases or division were observed.
since no cells in the metaphase, anaphase and telophase were counted in all tested concentrations when compared with that of the negative control where division until the telophase stage was evident. The mitotic index seen as the total number of dividing cells in cell cycle, with an increase (values higher than negative control indicate cell division) or decrease (values lower than negative control indicate alterations) of its value (Leme & Morales, 2009) were 79.61%, 34.03%, 67.54% and 83.73% on the negative control, 0.50%, 1.00% and 1.50% respectively. Based on this results, 0.50% and 1.00% concentrations showed values lower than the mitotic index of the negative control while at 1.50% concentration, mitotic index was greater. In terms of the mitotic index and cell phases observed, mitosis inhibition was observed at 0.50% concentration, because the mitotic index and early prophase cell counts were lower than the negative control and interphase cells respectively. The mitotic index of 1.00% concentration was also lower than the negative control however more cells were observed in the early prophase than non-dividing interphase cells, a similar finding at 1.50% concentration. The data observed between the significance of the 1.50% concentration pre- and post-treatment root length comparison stated earlier and its mitotic index 83.73% (a value closest to and even higher than the negative control) further supported that 1.50% concentration failed to inhibit mitosis, because at this concentration, a significant growth in root length and the highest mitotic index was observed. To sum this up, the common finding observed was the inhibition of mitosis until the early prophase stage on all concentrations tested, excluding the findings of the comparison of the pre- and post-treatment and mitotic index which is contrary to prior studies of Ozmen et al. (2007) and Bhattacharya & Haldar (2012) utilizing the same assay.

**CONCLUSION**

*Lansium domesticum* at 1.00% concentration inhibited mitosis until the 4-cell stage in the *T. gratilla* assay, and at the early prophase for *A. cepa* root tips at all concentrations tested with reduction of mitotic index at 0.50% concentration when compared to the negative control, but root lengths of pre- and post-treatment with the crude seed extracts were found insignificant in general except at 1.50% concentration. Antimitotic effect was observed better in the *A. cepa* assay but only qualitatively than with *T. gratilla* assay. Variations in the antimitotic activity of the crude seed extract on the two models of mitosis.
used in this study can be attributed to the inherent differences in the cell division of *T. gratilla* and *A. cepa*.

**RECOMMENDATION**

We recommend the isolation of specific phytochemicals in the *L. domesticum* and its assay for potential antimitotic activity. Partitioning of the crude extract into different fractions is also recommended.

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