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Preliminary Evaluation for Anti-Mitotic Activities of Aqueous *Piper sarmentosum* Roxb. Leaf Extract in *Allium fistulosum* Root Tip Cells

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ABSTRACT

Piper sarmentosum Roxb. is an herbaceous plant recognized for its pungent and aromatic smell. Numerous reports of the active phytochemicals, and bioactive properties were documented, however, studies specifically to the anti-mitotic activities of *P. sarmentosum* is yet limited due to the lack of direct information, comparative studies, and the involvement of plant models. The study aimed to perform a preliminary evaluation of the anti-mitotic activities of the aqueous leaf extract of *Piper sarmentosum* Roxb. in *Allium fistulosum* root tip cells. Roots of A. fistulosum were exposed to varying treatment concentrations *P. sarmentosum aqueous* extract (10%, 30%, 50%), distilled water served as the control group (0%). All root samples were processed and observed under a microscope. Anti-mitotic activities were evaluated based on the mitotic index and manifestation of mitotic abberations. The aqueous leaf extract of *P. sarmentosum* exhibited decreasing mitotic index relative to the increasing treatment concentrations (0% = 66.00%, 10% = 34.00%, 30% = 16.33%, 50% = 11.00%; p-value = 0.001). Manifestations of mitotic aberrations in *Allium fistulosum* root cells include chromosome adhesion, c-mitosis, chromosome bridging, chromosomal breakages, and binucleated cell. The results of the study provide additional information, and the need for validation in a larger sample is evident.

INTRODUCTION

The Philippines is a biodiverse rich country with a wide array of plant life that are geographically distributed throughout its islands. From the ancient times to the modern day period, and with the increasing knowledge and advancements of scientific technology, plants had been tested in the search for many bioactive properties that can be applied for nutraceutical, pharmaceutical, and medicinal uses. The attention of man to seek all possible potential from a certain plant continues to be sought after, in which one among them is *Piper sarmentosum* Roxb. *Piper sarmentosum* Roxb. is an herbaceous plant that belong to order *Piper*ales, family *Piper*aceae, genus *Piper* (Sun *et al.*, 2020). It has a wide distribution, found in Cambodia, China, India, Indonesia, Laos, Malaysia, the Philippines, and Vietnam (Raman *et al.*, 2012). Like all *Piper* species, *P. sarmentosum* is recognized for its pungent and aromatic smell (Rahman *et al.*, 2016). In terms of utilization, *P. sarmentosum* is used as a flavor additive to foods, and as an herbal medication for treating ailments such as fever, cough, flu, and rheumatism (Ware *et al.*, 2023).

Studies related to the phytochemical compositions of *Piper sarmentosum* Roxb. include essential oils, alkaloids, flavonoids, lignans and steroids; along with various bioactive properties such as anti-bacterial, anti-fungal, anti-inflammatory, anti-oxidative, anti-pyretic, hypoglycemic, insecticidal, and anti-cancer activities (Sun *et al.*, 2020).

However, studies in relation to the anti-mitotic activities exhibited by *P. sarmentosum* is yet limited. A gap seen is the lack of direct anti-mitotic studies of *P. sarmentosum* that involves the use of plants as the model organism, in which the meristematic root cells of *Allium* species are the most commonly utilized. Much of the published studies involved the use of mammalian cancer cell lines like HepG2, HeLa, HT-29 MCF-7, and V79 (Zainal Ariffin *et al.*, 2009; Sakilan *et al.*, 2019; Tammasakchai *et al.*, 2021). Furthermore, these studies involve a combination of cytotoxic, genotoxic, and anti-cancer properties, that usually includes but does not elucidate the anti-mitotic effects. Moreover, an investigation of potential mitotic aberrations in *Allium* root cells after treatment with *P. sarmentosum* has not yet been documented. In addition, there are limited comparative studies on the anti-mitotic activities of *P. sarmentosum* with other *Piper* species – specifically upon testing to *Allium* root cells.

Determining the anti-mitotic effects of *Piper sarmentosum* Roxb. in a model organism that is easily accessible, like *Allium* plants, could help fill the unknown spaces, especially on its profile down the cellular level – in which mechanisms can be compared and applied to other model systems from a higher order of organism (i.e. mammalian cell lines). Moreover, this can help advance the current scientific knowledge on the bioactive characteristics of *P. sarmentosum*.

This study generally aimed is to perform a preliminary evaluation to the anti-mitotic activities of *Piper sarmentosum* Roxb. aqueous leaf extract in *Allium fistulosum* root tip cells. Specifically, it aimed to: (i) determine the anti-mitotic effect of the aqueous leaf extract of *Piper*

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sarmentosum Roxb. based on the mitotic index, and (ii) identify the mitotic aberrations that manifested in the cells of *Allium fistulosum* roots exposed to the aqueous leaf extract of *Piper sarmentosum* Roxb.

LITERATURE REVIEW

Studies related to the anti-mitotic activities of *Piper sarmentosum* Roxb. were associated to the cytotoxic, genotoxic, and/or anti-cancer activities of the said plant. However, there are only a few reports that directly address to the anti-mitotic activities. From the study of Zairan Ariffin *et al.* (2009), human hepatoma cells (HepG2) treated with 10 µg/mL ethanolic extract of *P. sarmentosum* resulted to chromatin condensation, and fragmented nuclei – as observed in the cells subjected through acridine orange and ethidium bromide (AO/EB) staining. In addition, HepG2 cells treated in all concentration levels (subjected through gel electrophoresis) can induce nucleosomal DNA fragmentation. These outcomes infer how HepG2 cells treated with *P. sarmentosum* extract would be triggered to undergo cellular death through apoptosis. In the study of Tammasakchai *et al.* (2021), Chinese hamster lung fibroblast cells (V79) treated to the water extract of *P. sarmentosum* resulted to the formation of micronucleus; though the outcomes did not significantly increase the number of micronuclei to exhibit genotoxic effects the mentioned cell line. Despite of it, formation of a micronucleus is caused by fragments or a whole chromosome failed to migrate to the poles during the anaphase stage (Tammasakchai *et al.*, 2021). From this, it is considered that indicators and markers of anti-mitotic activities must occur during the M phase of the cell cycle. Generally, *Piper* species (including *P. sarmentosum*), contain the phytochemical *Piper*ine. Dias *et al.* (2021) conducted a study by utilizing pure *Piper*ine to test for cytotoxic and genotoxic effects in *Allium* cepa meristematic root cells. It was found that pure *Piper*ine exhibits cytotoxic and genotoxic effects in *Allium* cepa meristematic root cells. In addition, mitotic aberrations were observed which included micronucleus, nuclear bud, chromosomal breaking, binucleated cell, chromosomal adherence, c-metaphase, chromosomal loss, chromosomal bridge, multipolar anaphase, and nuclear alteration.

A few related studies on the anti-mitotic effects of other *Piper* species were also documented. From the study of de Assis Alves *et al.* (2023), the aqueous leaf extract of *Piper* anducum resulted to inhibitory effects, decreasing mitotic index, and indications of chromosomal changes such as chromosome lost, adherence, bridges, c-metaphase, and polyploidization, as tested in Lactuca sativa. Parmar & Parmar (2019) reported that the aqueous leaf extract of *Piper* chaba resulted in a significantly reduced mitotic index, and that mitotic aberrations were mostly observed in the prophase stage, as tested in Vigna radiata.

Hypothesis of the Study

There are no anti-mitotic activities of the aqueous leaf extract of *Piper sarmentosum* Roxb. in *Allium fistulosum* root tip cells.

MATERIALS AND METHODS

Preparation of Plant Extract and Treatment Concentrations

Fresh leaves of *Piper sarmentosum* Roxb. were obtained from Poblacion Norte Maddela, Quirino. Preliminary identification was based on its morphology, according to the descriptions from Raman *et al.* (2012). The leaves were thoroughly washed, air-dried to brittle, and ground to powder. Twenty grams (20g) of the ground plant sample was macerated in two hundred milliliters (200mL) distilled water. The aqueous extract was filtered, and treatment concentrations of 10%, 30%, and 50% were prepared through dilution with distilled water.

Allium fistulosum Root Tip Assay

The assay was performed according to the methods of Jose *et al.* (2020), and Krempels *et al.* (n.d.), with a few modifications. Locally available *Allium fistulosum* (green onion or scallion) were washed, and separated into individual stalks; the dried outer leaves unscaled, the leafy greens trimmed off, and the old roots removed but not to damage the root primordium at the base. The prepared A. fistulosum samples were suspended in individual test tubes with the base submerged in tap water, until roots have grown, but no longer than 2 to 3 centimeters in length. The samples were transferred to individual test tubes filled with the respective treatment concentrations, with one test tube filled with distilled water serving as the control. The duration of the treatment was for 24 hours. The roots were excised and transferred to separate vials filled with a fixative composed of 95% Ethanol and Glacial Acetic acid (3:1 v/v, respectively), and were fixed for 24 hours. The fixative was discarded and replaced with 70% aqueous ethanol for storage. The root tips were excised, gently warmed in 1N hydrochloric acid, stained with acetocarmine, and squashed on a glass slide topped with a cover slip. The slides were observed under a light microscope, and photomicrographs were obtained using a 5MP microscope camera (MC-D500U(E)).

Data Analysis

A total of 300 cells (100 cells per slide with 3 replicates) per treatment concentration were evaluated for anti-mitotic activities. Although it is recommended that 1000 cells per slide with 3 replicates per treatment sample should be analyzed (Nicuță *et al.* 2025), the cell count in this study was reduced due to time and sample constraints from the time this study was conducted. Despite of the reduced count, replicate slides were analyzed for each treatment to ensure data consistency and statistical reliability. The number of dividing and non-dividing cells were counted, and the mitotic index was computed using the formula (Jose *et al.*, 2020):

Presence of mitotic aberrations among the treatments were observed, recorded, and photographed. Identification for the type of mitotic aberrations were

$$Mitotic\ Index = \frac{Number\ of\ Diving\ Cells}{Total\ number\ of\ Cells}\ x\ 100$$

based from Dias *et al.* (2021).

Statistical Treatment of Data

The statistical treatment of the mean values among groups involved the use of One-way Analysis of Variance (ANOVA) with Duncan's Multiple Range Test to determine the significant differences between treatments ($p < 0.05$) (Ping *et al.*, 2012). The statistical methods were performed using the Statistical Package for the Social Sciences (SPSS) software (version 26).

RESULTS AND DISCUSSION

Table 1 presents the obtained mitotic index (MI) of *Allium fistulosum* root cells exposed to varying treatment concentrations of *P. sarmentosum* extract. At 10%, the MI was at 34.00%; at 30%, the MI was at 16.33%; and at 50%, the MI was at 11.00% – compared to the control (0%) with MI at 66.00%. Statistical analyses of the mean values (mitotic index) obtained a p-value of 0.001, which is less than 0.05, indicating significant difference among

Table 1: Mitotic Index in *Allium fistulosum* Meristematic Root Cells exposed to Varying Concentration Levels of *Piper sarmentosum* Roxb. Aqueous Leaf Extract.

Treatment Concentration	Number of Cells Observed	Number of Dividing Cells Undergoing Mitosis	Mitotic Index
50%	300	33	11.00 [a]
30%	300	49	16.33 [ab]
10%	300	102	34.00 [b]
0% (Control)	300	196	66.00 [c]

Sig. = 0.0001 ($p < 0.05$) ; superscripts of the same letter/s indicate no significant difference (DMRT)

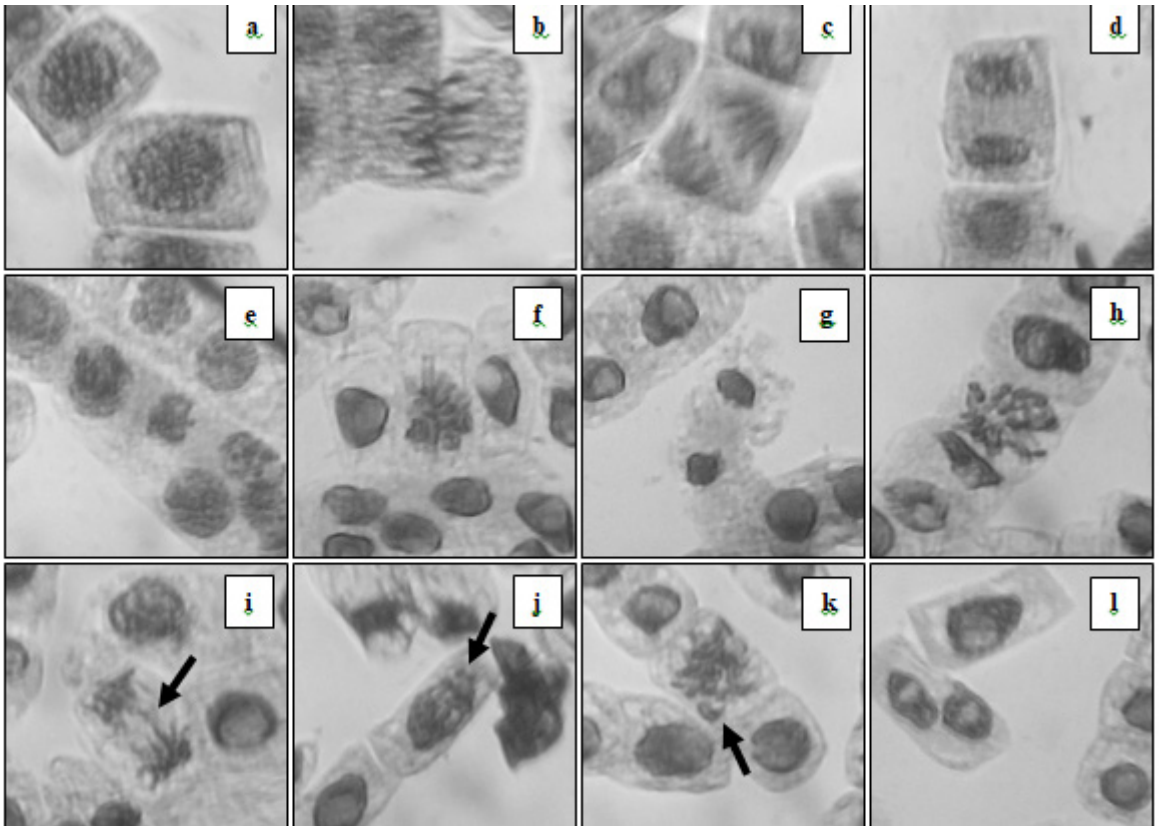

Figure 1: Photomicrographs of the normal mitotic stages and mitotic aberrations observed in *Allium fistulosum* root cells. [a-d] Normal mitotic phases (Prophase, Metaphase, Anaphase, Telophase);
[e-g] Chromosomal Adherence (Prophase, Metaphase, Late Anaphase); [h] C-metaphase;
[i] Chromosomal bridge; [j-k] Chromosomal breakage; [l] Binucleated cell

the mean values. The outcomes reflect that the mitotic index in the root cells of A. fistulosum decreases as the treatment concentration increases.

In addition, manifestation of mitotic aberrations was observed in the root cells of A. fistulosum exposed to the varying treatment concentrations (10%, 30% and 50%) of *P. sarmentosum* aqueous leaf extract. The observed mitotic aberrations include chromosomal adherence, c-metaphase, chromosomal bridge, chromosome breakage, and binucleated cell. Photomicrographs of the different mitotic aberrations, in comparison with the normal phases of mitosis, are shown in Figure 1. The findings of the manifested mitotic aberrations in this study have similarities to some of the results from Dias *et al.* (2021). The molecular context of anti-mitotic activities suggests that it can be due to increasing oxidative stress by reactive oxygen species (ROS), which increases the risk of DNA damage, including poorly repaired damage during cell division, which can lead to mutations (Dias *et al.*, 2021). The concepts can be directly linked to the study, that exposure of the meristematic root cells of A. fistulosum to the aqueous leaf extract of *P. sarmentosum* affected the mitotic activities, and chromosomal formation and integrity.

Although the number of cells analyzed in this study was lower than the standard recommendation, this design was adopted due to the resource-limited nature of the investigation. The goal was to gather preliminary information about the anti-mitotic activities of the aqueous extract of *Piper sarmentosum* Roxb., instead of conducting an extensive and comprehensive evaluation. The observed patterns of mitotic inhibition and chromosomal abnormalities still offer a reliable indication of the plant's potential activity. This calls for additional research with larger sample sizes for validation.

CONCLUSIONS

From the obtained findings and set of records, the aqueous leaf extract of *Piper sarmentosum* Roxb. was determined to bear anti-mitotic activities in *Allium fistulosum* root tip cells, wherein the mitotic index decreases relative to the increasing treatment concentrations. Additionally, manifestations of mitotic aberrations - particularly chromosome adhesion, c-mitosis, chromosome bridging, chromosomal breakages, and binucleated cell, were observed. The results of the study shall provide additional information, though further exploration and validation to larger sample size is highly regarded.

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