Antiproliferative effects and genotoxicity of hydroethanolic extracts of Plumbago zeylanica (L.)

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Abstract

Background

Plumbago zeylanica is a shrub used in traditional medicine to cure a variety of human and animal diseases. Additionally, this plant species is employed to control mites in agricultural plantings. Communities have reported instances of toxicity impacting small livestock's embryonic development. Cellular stress or genotoxic effects are frequently the cause of severe degenerative illness and may accompany this cytological activity. This study aims to assess how hydroethanolic extracts of *P. zeylanica* affect cell cycle and proliferation in relation to chromosomal mechanisms.

Methods

The antiproliferative activity of *P. zeylanica* extracts was evaluated using the *Saccharomyces cerevisiae* model coupled with methylene blue staining. The genotoxicity of the extracts was performed using the *Allium cepa* model. Twelve *Allium cepa* bulbs were grown hydroponically for 48 hours in pots filled with distilled water such that the base of the main root was immersed in the liquid. The bulbs were then moved to different solutions of *P. zeylanica* extracts to be examined (20 mg/ml) for 24 hours. Positive controls included colchicine and cyclophosphamide.

Results

Significant dose-dependent cytotoxicity of *P. zeylanica* root and leaf extracts on *S. cerevisiae* was found by the antiproliferative assay. The extract concentrations (IC_{50}) that killed 50% of the yeast were 2.74 mg/ml and 3.09 mg/ml, respectively. After a 24-hour treatment, both extracts significantly inhibited mitosis and resulted in chromosomal and nuclear abnormalities in *Allium cepa* root meristematic cells. The corresponding mitotic indices were 48.95 ± 0.53% for the leaf extract and 33.42 ± 1.24% for the root extract. Similarly, cells treated with root extract showed a substantial increase in binucleated cells (0.98 ± 0.01%), chromosome agglutinations (33.90 ± 0.57%), elongated cells (0.83 ± 0.00%), chromosome fragmentation (1.16 ± 0.07%), giant cells (2.17 ± 0.19%), and equatorial plate disorganization (0.17 ± 0.0%). The primary cytological abnormalities found in cells exposed to *P. zeylanica* leaf extract were chromosome agglutination (26.45 ± 0.34%), binucleated cells (0.13 ± 0.00%), elongated cells (0.34 ± 0.01%), and giant cells (1.67 ± 0.03%).

Conclusions

These two extracts of *P. zeylanica* showed cytotoxicity, significant cell cycle suppression and cytogenetic abnormalities. These markers of toxicity suggest that the *P. zeylanica* leaf and root extracts may have antiproliferative and genotoxicological effects.

Background

Plants have traditionally been used in various societies and are often considered natural goods. They are sometimes perceived as safe for human and animal consumption. Medicinal recipes derived from plants are increasingly used as complementary therapies, especially for patients with chronic illnesses [1]. Plants provide a wide range of compounds with various pharmacological actions that can enhance population quality of life [2, 3]. Despite the various advantages of plant extracts, some of them may contain bioactive substances, such as glycosides, alkaloids and flavonoids, that may have various effects, including genotoxic activity [4, 5, 6]. Some compounds produced by secondary plant metabolism are poisons or enzyme disruptors, which can affect the body's physiological equilibrium or induce spontaneous damage to endogenous macromolecules [7, 8, 9].

In Africa, biodiversity offers a vast therapeutic resource. Among these is *Plumbago zeylanica*, a herbaceous plant often found in sunny areas and dry forests [10, 11]. This species of Asian and African flora is used in traditional medicine for its many therapeutic benefits. It is known to combat mites, dermatophytosis and tumors. However, cases of embryonic toxicity in small livestock and keratin cells in humans have been reported in communities [10, 11]. Indeed, fresh crude extracts of *P. zeylanica* are highly vesicant and cytotoxic. Their bioactive effect appears to significantly affect cell proliferation, hence the use of *P. zeylanica* to combat mites, helminths, nematodes and dermatophytes [11]. The increasing use of recipes based on *P. zeylanica*, especially in cosmetic preparations, raises concerns about its potential effect on human cell cycle dynamics. Thus, the objective of this study is to evaluate the toxicity and cytogenetic impact of hydroethanolic extracts from the leaves and roots of *P. zeylanica*.

Methods

Plant material

The plant material consisted of leaves and roots of *P. zeylanica*. These organs were collected in Avétonou (Agou area) in May 2023.

Extraction

The plant parts were carefully washed, cut into small pieces and dried in the laboratory (LaSBASE) under air conditioning for two weeks. These plant parts were then finely ground in an electric mill [12]. Extraction was carried out by macerating 100 g of dry powder from each plant organ in one liter of hydroethanolic solution under continuous stirring for 72 hours at room temperature. The macerate obtained was filtered twice on cotton wool and then once on Whatman paper. The filtrate was finally evaporated under vacuum at 40°C and at reduced pressure between 999–1000 in a rotary evaporator [13].

Evaluation of the antiproliferative effect of extracts

Saccharomyces cerevisiae model

The antiproliferative effect of *P. zeylanica* extracts was evaluated using the *Saccharomyces cerevisiae* assay. This method uses *Saccharomyces cerevisiae* as a eukaryotic cell model for cancer research to study fundamental cellular mechanisms based on the genetic similarities between this yeast and eukaryotic cells [14].

Preparation of Saccharomyces cerevisiae culture medium

PDB (Potato Dextrose Broth) medium was used for the growth of *Saccharomyces cerevisiae*. This medium was performed as follows: 200 g of peeled and chopped potatoes were boiled in 200 ml of distilled water for 30 min. The mixture was filtered. 10 g of glucose were added to the collected filtrate, and the volume was adjusted to 1000 ml with distilled water [14]. The resulting broth was divided into several bottles and autoclaved for 20 minutes at 120°C to eliminate any contamination by saprophytic germs.

Preparation of Saccharomyces cerevisiae inoculum

Two grams (2 g) of commercial *Saccharomyces cerevisiae* yeast were inoculated into 125 ml of sterile PDB (Potato Dextrose Broth) medium, then incubated at 30°C for 24 hours [14].

Experimental protocol

Three sets of 6 tubes, each containing 2.5 ml of sterile PDB culture medium, were added with 250 µl of yeast inoculum with an optical density of 0.1 at 650 nm. Hydroxyurea (HYDREA) was used as a positive control [14, 15]. A hydroxyurea solution and hydroethanolic *P. zeylanica* leaf and root extracts were respectively applied to each of these three sets of tubes. Each solution's ultimate concentrations were C0 = 0.62 mg/ml, C1 = 1.2 mg/ml, C2 = 2.5 mg/ml, C3 = 5.0 mg/ml, C4 = 10.0 mg/ml and C5 = 20.0 mg/ml. A negative control was performed with distilled water.

Each tube was incubated at 30°C for 24 hours. Following this time, each tube was filled with 0.5 ml of 0.1% methylene blue. Living cells stay colorless, whereas dead cells turn blue [14]. Under an optical microscope, a Malassez cell was used to count living and dead cells [16]. Finally, the following formula was used to assess each *P. zeylanica* extract's percentage of cytotoxicity:

$$\text{Cytotoxicity } (\%) = \frac{\text{Number of dead cells}}{\text{Number of viable cells} + \text{Number of dead cells}} X100$$

Evaluation of the genotoxicity of leaf and root extracts of P. zeylanica

Allium cepa model

Allium cepa (2n = 16) [17], commonly known as onion, is a herbaceous monocotyledonous plant belonging to the Alliaceae [18, 19]. Grown in many countries for culinary purposes. This plant has a karyotype consisting of five pairs of chromosomes with centromeres located in the median to submedian position. Two pairs of chromosomes have submedian centromeres, and one pair is characterized by the presence of satellite chromosomes [20]. *Allium cepa* is a model that allows toxicity to be assessed based on root elongation, mitotic index and germination [16]. From a genotoxicological perspective, this species can be used to evaluate the frequency of micronuclei and chromosomal abnormalities in root tips and to perform comet assays using appropriate protocols [21].

Antimitotic test

Bulbs of similar diameters are selected, then cleaned and rinsed. The old roots and the first layer of skin are removed. The base of the bulb was cut slightly to promote the growth of future roots [22, 16]. For this toxicity test, 12 bulbs are then placed in hydroponic culture in 200 ml pots filled with distilled water so that the base of the main root is immersed in the liquid. This incubation is carried out for 48 hours in distilled water, then transferred to different solutions of *P. zeylanica* extracts (20 mg/ml) for 24 hours. Two positive controls consisting of roots in contact with colchicine (1 mg/ml) and cyclophosphamide. The negative control was also performed with distilled water [23].

For each bulb, six roots were collected and fixed in the mixture (ethanol/acetic acid, 3/1) for 24 hours and transferred to tubes containing 70% ethanol and stored at 40°C. The roots were then stained with acetic carmine (45%) to obtain a good contrast between the chromosomes and the colourless cytoplasm. Observations were made under an optical microscope to determine the number of dividing cells. For each extract, three apices were examined and 350 cells analyzed [23] to detect changes in chromatin structure, count the phases of mitosis, and identify chromosomal and nuclear abnormalities [16].

Mitotic index (MI) assessment

The mitotic index represents the percentage of cells undergoing division at a given time. It assesses proliferative capacity by counting mitoses in a tissue preparation. This index consists of counting the percentage of cells arrested in mitosis out of the total number of cells examined [24].

$$\mathrm{MI}\ (\text{Mitotic index }\%) = \frac{\text{Number of dividing cells } (\mathrm{P}+\mathrm{M}+\mathrm{A}+\mathrm{T})}{\text{Number of cells examined}} X100$$

P: prophase; M: metaphase; A: anaphase; T: telophase.

Phase index (PI) evaluation

This index is calculated to determine the percentage of cells in each phase of mitosis (prophase, metaphase, anaphase and telophase) [25, 26].

$$\mathrm{PI}\ (\text{Phasis index}\ \%) = \frac{\text{number of cells in a single phase}(\mathrm{P}, \mathrm{M}, \mathrm{A}, \mathrm{T})}{\text{Number of cells examined}} X100$$

Evaluation of the cytotoxicity limit value (CLV)

The cytotoxicity limit value of *P. zeylanica* extracts is calculated using the following formula [26]:

$$\mathrm{VLC}\ (\%) = \frac{\text{MI of treated cells}}{\text{MI of control cells}} X100$$

Chromosomal and nuclear abnormality testing

Several types of chromosomal aberrations are considered at different stages of the cell cycle [25, 26]. Clastogenic abnormalities include bridges and chromosome breaks, while physiological abnormalities include c-mitosis, lagging chromosomes and chromosome condensation [24]. Nuclear abnormalities are characterized by morphological alterations in interphase nuclei resulting from the action of cellular exposure to a genotoxic agent. Micronuclei are acentric chromosome fragments excluded from the daughter cell nucleus during cell division (clastogenic phenomenon) [22]. These various abnormalities were examined with the determination of the mitotic index by optical microscope observation of cells from the root meristematic apices of *Allium cepa* after exposure to *P. zeylanica* extracts. The aberration index is calculated as follows [14]:

$$\mathrm{AI}\,(\text{Aberration index}\ \%) = \frac{\text{Total chromosomal aberrations}}{\text{Total cells examined}} X100$$

Polyploidy test coupled with root elongation

Twelve (12) bulbs were grown hydroponically at room temperature (25–30°C) for 48 hours in 200 ml pots containing distilled water and transferred daily to pots containing fresh solutions of *P. zeylanica* root and leaf extracts (20 mg/ml), colchicine (10 mg/ml) and cyclophosphamide (10 mg/ml). Five (5) days after the start of exposure, root elongation was calculated by measuring the roots before and after exposure to the different solutions in order to determine whether longer exposure to *P. zeylanica* extracts could produce even more genotoxicological effects [27]. Three bulbs were used for each solution, and three roots per bulb were identified for the root elongation test. The size of these identified roots per bulb was measured at the beginning and end of exposure to the different solutions. Root morphology and growth were examined from each bulb [22, 24].

Data analysis

The data were analyzed using analysis of variance (ANOVA). This analysis was facilitated using Graphpad Prism 8.4.3 software with a 95% confidence interval.

Results

Antiproliferative effect of hydroethanolic leaf and root extracts of P. zeylanica

The observations showed that exposure of *Saccharomyces cerevisiae* to hydroethanolic extracts of *P. zeylanica* during 24 hours resulted in notable morphological changes associated with cell death in cells subjected to the various treatments compared to control cells. Similarly, our observations also showed viable *Saccharomyces cerevisiae* cells to be colourless, while dead cells appeared blue under the optical microscope (Fig. 2).

Growth inhibition and a significant dose-dependent decrease in cell viability were revealed in cells exposed to hydroethanolic leaf and root extracts of *P. zeylanica* ($p < 0.0001$). At a concentration of 5 mg/ml (C3), the cytotoxicity percentages were 51.99% for hydroxyurea, 78.68% for the root extract, and 66.01% for the hydroethanolic extract of *P. zeylanica* leaves. The extract concentrations that cause the death of 50% of *Saccharomyces cerevisiae* yeast (IC_{50}) were determined from the linear regression curves $Y = 16.84X + 7.898$ for hydroxyurea, $Y = 20.14X + 16.63$ for the root extract, and $Y = 22.33X + 4.797$ for the leaf extract of *P. zeylanica*. These IC_{50} concentrations were 3.54 mg/ml for hydroxyurea, 2.74 mg/ml for root extract, and 3.09 mg/ml for leaf extract. No significant differences were observed between the IC_{50} of the different solutions tested at a concentration of 5 mg/ml (Fig. 3).

Toxic effect of P. zeylanica extracts on the mitotic index

Micrographs of root apex tissues from untreated *Allium cepa* (controls) showed a homogeneous organization of cells arranged in a palisade pattern. Our observations also revealed the presence of root meristematic cells in prophase (P), metaphase (M), anaphase (A), and telophase (T) within these tissues. This observation allowed us to validate the *Allium cepa* model adopted for the rest of our work (Fig. 4).

The analyses revealed a significantly lower proportion of dividing cells in *Allium cepa* root meristem cells exposed to *P. zeylanica* extracts compared to untreated *Allium cepa* root apices (Fig. 5). Similarly, significant differences between the mitotic index observed in root meristematic tips exposed to *P. zeylanica* root extract (33.42 ± 1.24%) and that recorded in cells exposed to leaf extract (48.95 ± 0.53%) ($p < 0.0001$) were observed. The mitotic index recorded in cells treated with colchicine (34.28 ± 0.49%) showed no significant differences when compared to that recorded in cells treated with cyclophosphamide (31.95 ± 0.96%).

Toxic effect of extracts on the cytotoxicity limit value

A significant difference between the cytotoxicity limit value (CLV) was observed in cells treated with leaf extract (57.81 ± 0.96%) and that recorded in cells exposed to root extract (39.47 ± 2.38%) of *P. zeylanica* ($p < 0.0001$). Furthermore, the cytotoxicity threshold value of *Allium cepa* cells treated with root extract showed no significant variation compared to those observed in cells exposed to colchicine (40.49 ± 0.82%) and cyclophosphamide (37.73 ± 1.48%).

Toxic effect of extracts on the phase index

Table 1
Toxic effect of *P. zeylanica* extracts on the phase index

Treatments	Concentration (mg/ml)	IPR (%)	IME (%)	IAN (%)	ITE (%)
Control	-	57.71 ± 0.18	1.43 ± 0.36	7.43 ± 0.13	18.09 ± 0.14
Colchicine	1.00	1.71 ± 0.80	30.00 ± 0.36	0.00 ± 0.00	2.57 ± 0.41
Cyclo	1.00	31.71 ± 0.3	0.00 ± 0.00	0.00 ± 0.00	1.15 ± 0.50
Exth Ra	20.00	26.29 ± 0.6	1.42 ± 0.23	0.29 ± 0.27	4.42 ± 0.01
Exth Fe	20.00	38.66 ± 0.54	2.00 ± 0.15	0.86 ± 0.80	7.43 ± 0.30

Cyclo: cyclophosphamide; IPR: prophase; IME: metaphase; IAN: anaphase; ITE: telophase; Exth Ra: roots extract; Exth Fe: leaves extract; Cyclo: cyclophosphamide

Toxic effect of extracts on chromosomes and cell nuclei

The observations showed the appearance of chromosomal and nuclear abnormalities in the root meristematic cells of *Allium cepa* exposed to the hydroethanolic extracts of *P. zeylanica* examined. These abnormalities were mainly chromosome agglutinations (33.90 ± 0.57%), followed by binucleated cells (0.98 ± 0.01%), elongated cells (0.83 ± 0.00%), chromosome fragmentation (1.16 ± 0.07%), giant cells (2.17 ± 0.19%), and equatorial plate disorganisation (0.17 ± 0.0%). These data reveal a significant difference between the total indices of chromosomal and nuclear abnormalities observed in cells exposed to root extract and those exposed to leaf extract of *P. zeylanica* compared to cells ($p < 0.0001$).

Table 2
Toxic effect of extracts on the index of chromosomal and nuclear abnormalities

Extracts	AC ± SEM (%)	CB ± SM (%)	AL ± SM (%)	FR ± SEM (%)	DP ± SEM (%)	CG ± SEM (%)	PC ± SEM (%)	Total (%)
Control	2.00 ± 0.33	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.00 ± 0.33
Colchicine	24.85 ± 0.59	0.71 ± 0.06	1.24 ± 0.02	3.73 ± 0.01	0.00 ± 0.00	0.19 ± 0.00	0.00 ± 0.00	17.72 ± 0.68
Cyclo	17.90 ± 0.49	0.64 ± 0.03	0.61 ± 0.08	1.71 ± 0.12	0.93 ± 0.01	0.51 ± 0.01	0.46 ± 0.00	10.88 ± 0.74
Exth Ra	33.90 ± 0.57	0.98 ± 0.01	0.83 ± 0.00	1.16 ± 0.07	0.17 ± 0.00	2.17 ± 0.19	0.00 ± 0.00	27.72 ± 0.84
Exth Fe	26.45 ± 0.34	0.13 ± 0.00	0.34 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	1.67 ± 0.03	0.00 ± 0.00	18.37 ± 0.38

CG: giant cells; CB: binucleated cells; FC: chromosomal fragmentation; AC: chromosomal agglutination; PC: chromosomal bridges; AL: elongated cells; DP: disruption of the equatorial plate; Exth Ra: hydroethanolic extract from roots; Exth Fe: hydroethanolic extract from leaves; Cyclo: cyclophosphamide.

Toxic effect of treatment with P. zeylanica extracts on root elongation and polyploidy in the root apices of Allium cepa

The results revealed that the growth (size) of *Allium cepa* roots treated with *P. zeylanica* root extracts decreased significantly compared to the controls (p□0.001).

Table 3
Toxic effect of *P. zeylanica* extracts on root elongation

Treatments	Concentration (mg/ml)	Root elongation (mm)
Control (negative)	-	38.67 ± 0.12
Colchicine	10 mg/ml	24.00 ± 0.10
Cyclophosphamide	10 mg/ml	27.71 ± 0.21
Root extract	20 mg/ml	29.33 ± 0.17
Leaf extract	20 mg/ml	34.27 ± 0.24

The root meristem of *Allium cepa* treated with hydroethanolic extracts from *P. zeylanica* roots and leaves, as well as with cyclophosphamide and distilled water, exhibited a tapering cap on its apices. Additionally, the majority of roots exposed to colchicine (es5) showed no further growth in length, while our observations revealed the development of tumors from the root meristem.

Discussion

The generation of new cells is one of the most fundamental aspects of cell biology and life. To this end, proper regulation of the cell cycle is essential to human and animal health, as highlighted by numerous diseases associated with errors in cell cycle regulation [28, 29]. Genotoxicity testing is therefore particularly important when using plant extracts for medicinal, food, cosmetic, or pharmaceutical purposes in order to guarantee product safety. To this end, *Saccharomyces cerevisiae* is an excellent eukaryotic model frequently used to elucidate the biological processes of life involving cell division phenomena and allows the screening of chemicals for the prediction of toxicity in animals or humans [30, 31]. This yeast was used to evaluate the antiproliferative activity of hydroethanolic extracts from the leaves and roots of *P. zeylanica*. We used hydroxyurea, known for its cytotoxic effect, as a reference.

According to our findings, *Saccharomyces cerevisiae* exposed to the extracts for 24 hours showed necrosis and a significant dose-dependent cell mortality ($p < 0.0001$) (Fig. 2). Other authors have reported similar outcomes [32]. Indeed, crude extracts from the foliar mycoendophytes of *Peganum harmala* revealed marked cytotoxicity on *Saccharomyces cerevisiae* cells [14]. This antiproliferative effect could be attributed to the presence of cytotoxic compounds in the extracts examined [33]. The potential action of these molecules at the mitochondrial level via the inhibition of succinate dehydrogenase or the opening of permeability pores due to increased calcium ions in the cytosol would lead to the disorganization of cell membranes, followed by the release of cytochrome C, excessive generation of oxidative free radicals, and intracellular ATP depletion [34]. This would induce the loss of cell viability through apoptosis or autophagy [35]. These findings on *Saccharomyces cerevisiae* prompted an assessment of *P. zeylanica* extracts' genotoxicity in order to investigate their interference with cytogenetic mechanisms and the normal progression of the cell cycle in *Allium cepa* root meristem cells [36].

A significant reduction in the mitotic index ($p < 0.0001$) in treated *Allium cepa* root meristem cells compared to controls was revealed. This suggests that hydroethanolic extracts of *P. zeylanica* inhibit normal cell cycle progression, confirming its use in traditional medicine to treat neoplastic diseases [11, 37]. These data are similar to those reported by other authors in the literature [38]. In a study, the methanolic extract of *Peganum harmala* leaves led to a significant reduction in the mitotic index of *Allium cepa* root meristem cells [25]. This inhibition of mitosis observed in *Allium cepa* root meristem cells led to the analysis of the different phases of the cell cycle in order to detect the impact of the extracts on each stage of cell division.

Phase indices in cells exposed to the extracts showed a significant regression, according to the findings. Cytotoxicity limit values decreased as a result ($p < 0.0001$). This regression in mitotic indices below 50% compared to controls reflects a mitodepressive state, confirming the antimitotic effect of the extracts studied [39]. This effect could be attributable to the bioactive compounds (phenolic and terpenic) present in the extracts examined [40]. Secondary metabolites are known to inhibit cyclins and cyclin-dependent kinases (CDKs), but also to modulate the expression of enzymes involved in cell growth and

proliferation [29]. In fact, gallic acid is known to reduce the proliferation of various human cancer lines. In human leukemia cells, it causes cell cycle arrest in the G0/G1 phase associated with a decrease in cyclin D and E levels [30, 31]. Plumbagin isolated from *P. zeylanica* is known for its significant antiproliferative effects on human papillary thyroid cancer cells by promoting apoptosis, modulating the cell cycle, and limiting invasion and proangiogenic properties [41, 42]. In general, antimitotic substances interfere with cellular enzymatic processes through mimicry or with the structural and functional dynamics of DNA (cleaving agents, alkylating agents), causing cytogenetic disorders [32, 43, 44]. We therefore conducted genotoxicity tests to assess the effect of the extracts on chromosome physiology and structure.

Observations show the presence of fragmented chromosomes, giant cells, disorganised equatorial plates in metaphase, interchromosomal bridges, a marked tendency toward chromatin agglutination, and the presence of binucleated cells. These findings suggest the potential effect of the *P. zeylanica* extracts (20 mg/ml) examined on the genetic material of *Allium cepa* root meristem cells. Thus, exposure of *Allium cepa* meristem roots to *P. zeylanica* extracts may have generated oxidative stress capable of causing chromatin precipitation or led to the failure of mitotic enzymes, resulting in dysfunction of DNA replication processes [45]. These findings support previous work that has highlighted the cytotoxic and genotoxic effects of phytochemicals found in some medicinal plants [46]. In numerous studies, plumbagin has induced oxidative stress and caused cytogenetic disorders in certain cancer cell lines [47].

The presence of giant cells suggested the dysfunction of the cytoskeleton, resulting in an inflexible cellular structure [48, 49]. This would be the cause of endoreduplication without cell division [23]. This is confirmed by the detection of elongated, gigantic binucleated cells or cells with lobed nuclei after treatment (Fig. 41). Therefore, the extracts under investigation would have disrupted the cytoskeleton and resulted in polyploidy through DNA duplication without cell division [23, 50].

Meristem is the origin of all root cells [16, 22]. Structural or cytogenetic abnormalities within meristematic cells could result in harmful or deleterious manifestations in young shoots or lead in the long term to mutagenesis or cellular ageing [51, 52]. Overall, the mitotic and nuclear abnormalities observed reflect a state of mitotic catastrophe induced by exposure to *P. zeylanica* extracts [23, 45, 53, 54]. It was therefore important to verify whether prolonged exposure of *Allium cepa* root meristem cells to the extracts examined did not lead to more significant genotoxicological effects.

Polyploidy assay performed in conjunction with root elongation revealed a significant decrease in the size of *Allium cepa* roots treated with both *P. zeylanica* extracts compared to control cells (p□0.001). This confirms the mitodepressive effect of these extracts. Macroscopic observation of root tips showed root tumors in roots treated with colchicine, while there was no polyploidy (tumors) in cells treated with cyclophosphamide and *P. zeylanica* extracts. The absence of tumors in cells treated with plant extracts suggests that they act on the cell cycle through a mechanism other than that observed in cells treated with colchicine. These results are similar to those of other authors who have reported that

phytoconstituents, by binding to cellular macromolecules, may sometimes limit cell cycle progression with marked effects on root growth or generate stress that triggers cell death pathways [25].

Conclusion

The antiproliferative activity of hydroethanolic extracts from the leaves and roots of *P. zeylanic*a was evaluated using the *Saccharomyces cerevisiae* assay. The hydroethanolic root and leaf extracts of *P. zeylanica* showed significant cytotoxicity ($p < 0.0001$) on the growth of *Saccharomyces cerevisiae* cells with IC_{50} inhibitory concentrations slightly lower than those revealed by hydroxyurea. Genotoxicity tests performed with *P. zeylanica* extracts revealed significant suppression of the mitotic index as well as chromosomal and nuclear aberration indices, suggesting that the extracts examined may have adverse effects on the normal progression of the cell cycle in *Allium cepa* root meristem cells. The treatments resulted in a significant decrease (p□0.001) in root elongation of *Allium cepa* meristematic apices, confirming the mitodepressive effect of the extracts examined. In summary, using *P. zeylanica* extracts for therapeutic purposes requires greater caution to avoid any molecular or cellular harm.

Abbreviations

A
Anaphase
AC
Chromosome agglutination
ADN
Deoxyribonucleic acid
AL
Elongated cells
ARN
Ribonucleic acid
ANOVA
Analysis of variance
ATP
Adenosine triphosphate
CB
Binucleate cells
CDK
Cyclin-dependent kinases
CG
Giant cells
Colch
Colchicine

Cyclo
Cyclophosphamide
DP
Disorganization of the equatorial plate
Exth Fe
Hydroethanolic extract from the leaves
Exth Ra
Hydroethanolic extract from the roots
FC
Chromosome fragmentation
IA
Chromosomal aberration index
IAN
Anaphase index
IM
Mitotic index
IME
Metaphase index
IP
Phase index
IPR
Prophase indices
ITE
Telophase index
M
Metaphase
P
Prophase
PDB
Potato Dextrose Broth
T
Telophase
VLC
Cytotoxicity limit value

Declarations

Ethical approval and consent to participate

Not applicable

Consent for publication

Not applicable

Conflicts of interest

The authors declare that they have no competing interests.

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Author Contribution

E.T., Y.H. and P.P. participated in the conceptualisation of the project, the development of the methodology, the performance of laboratory tests, data analysis, and the writing of the original manuscript. K.E.T. participated in the preparation of plant extracts. N.S.R.K. participated in conducting laboratory tests. S.K.D. offered advice on selecting the plant and took part in the supervision of work and proofreading of the manuscript. T.T. participated in conceptualisation, methodology validation, supervision of work, writing, and proofreading of the manuscript. All authors read and approved the final manuscript.

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Data Availability

The data from this study are available from the corresponding author upon reasonable request.

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Figures

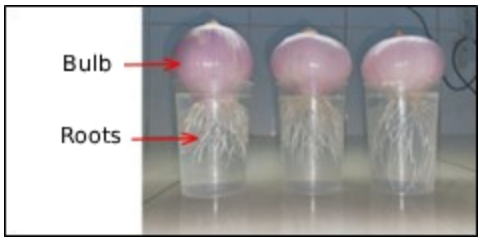

Figure 1

Young shoots of *Allium cepa.*

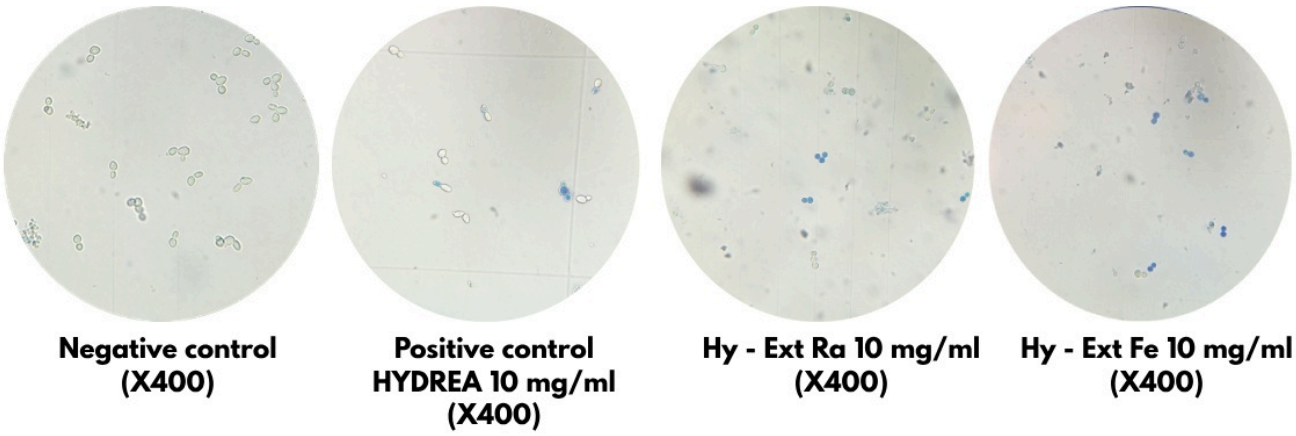

Figure 2

Micrograph of *Saccharomyces cerevisiae* cells exposed to *P. zeylanica* extracts. Exth Ra: hydroethanolic extract of the roots; Exth Fe: hydroethanolic extract of the leaves; hydroxyurea is used as a positive control; magnification: x400(images from own research).

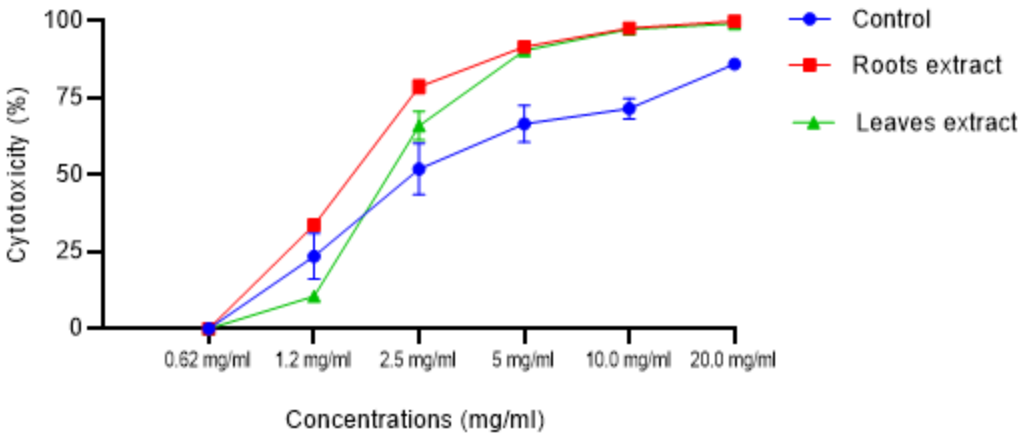

Figure 3

Variation in cytotoxicity of extracts at different concentrations. Each value is expressed as a percentage ± 95% confidence interval.

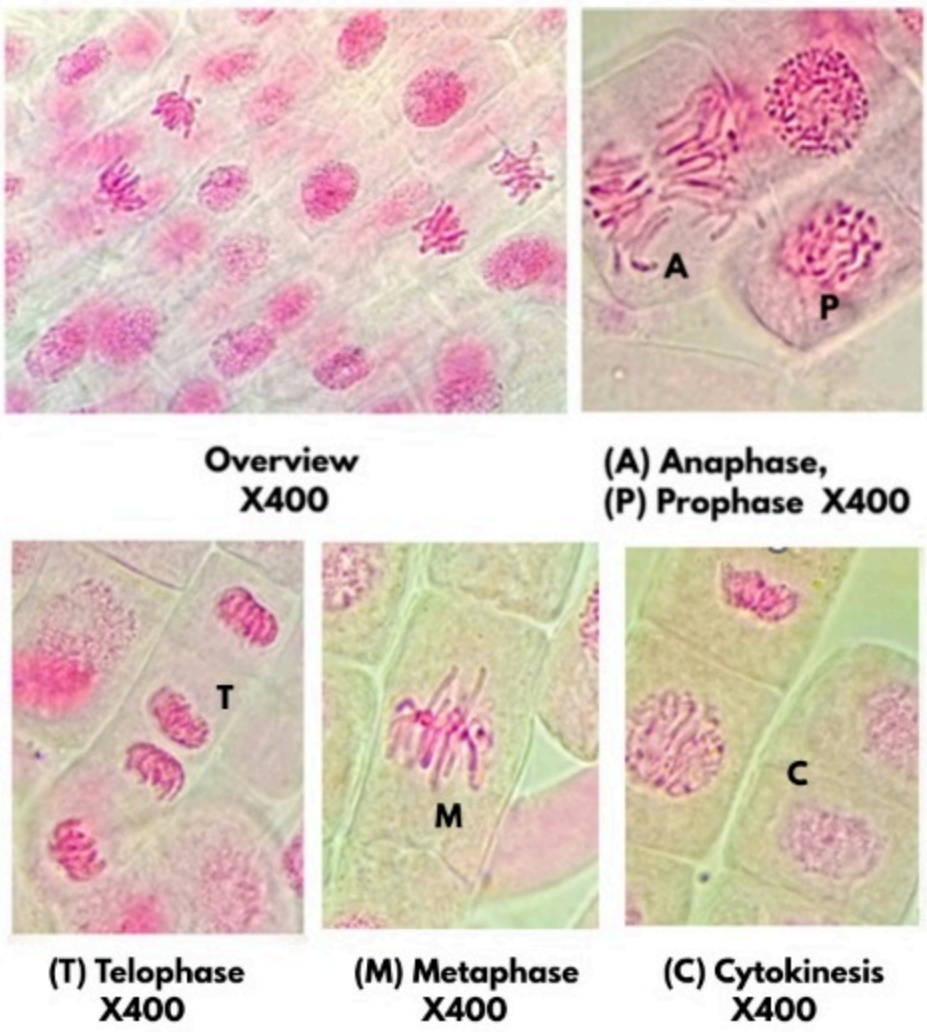

Figure 4

Micrograph of dividing meristematic cells of *Allium cepa*. Acetic carmine staining; magnification: x400 (images from own research).

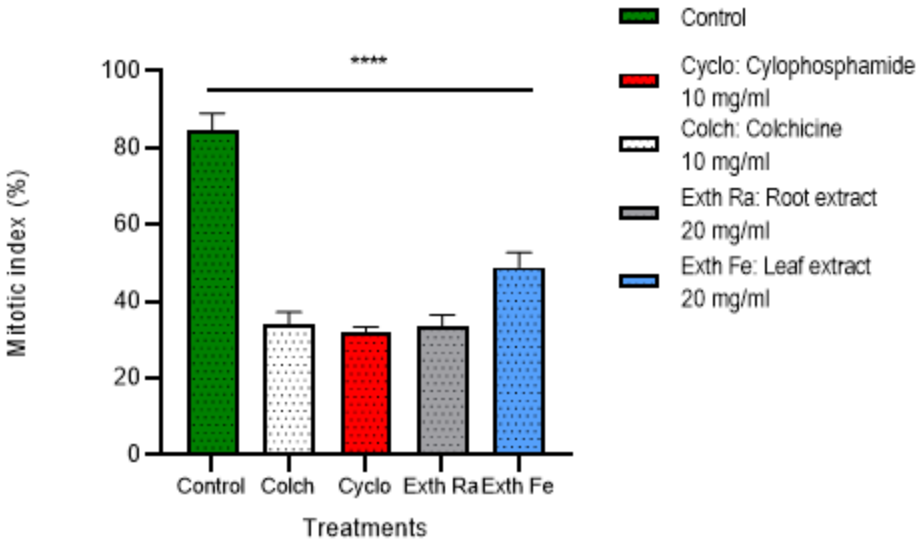

Figure 5

Variation in mitotic indices in meristematic cells of *Allium cepa*. Colchicine is used as an aneugenic positive control; cyclophosphamide is used as a clastogenic positive control. Each value is expressed as a percentage ± 95% confidence interval.

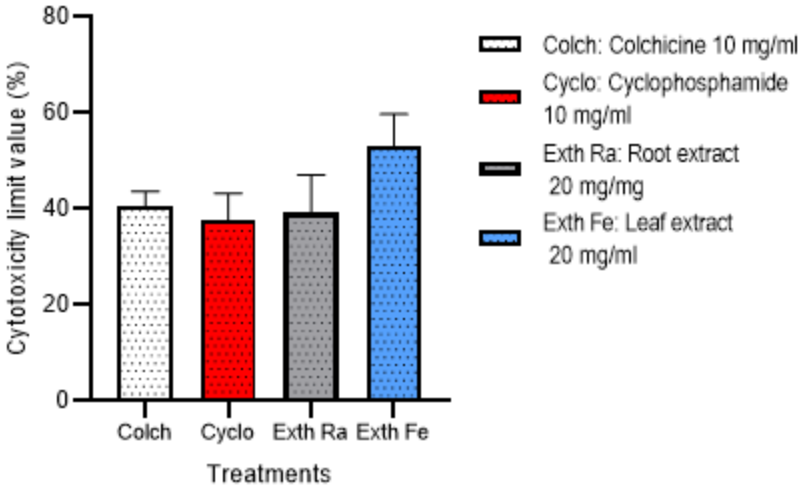

Figure 6

Variation in cytotoxicity limit values in root meristematic cells of *Allium cepa*. Colchicine (Colch) is used as an aneugenic positive control; cyclophosphamide (Cyclo) is used as a clastogenic positive control. Each value is expressed as a percentage ± 95% confidence interval.

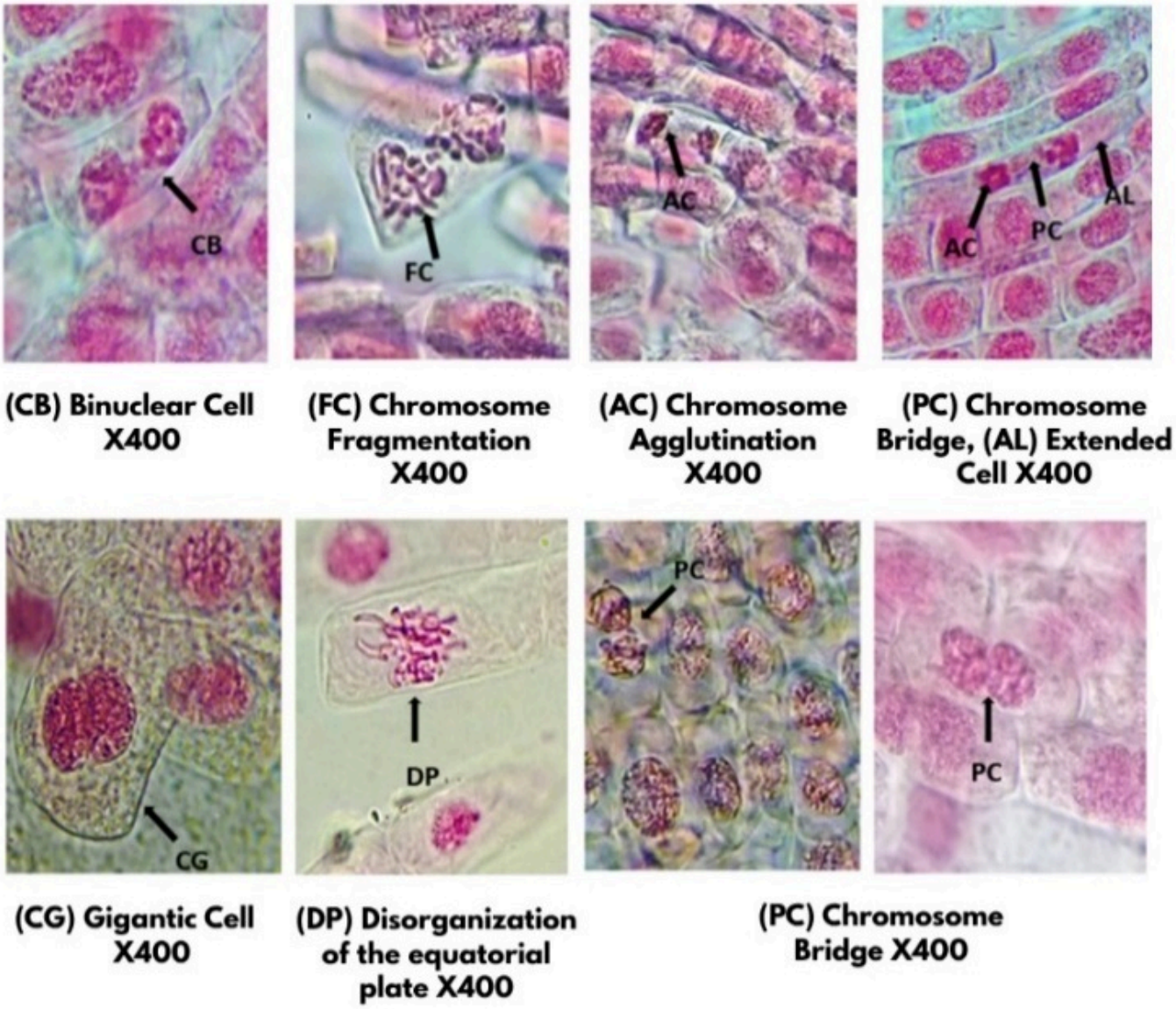

Figure 7

Micrograph of *Allium cepa* root cells exposed to *P. zeylanica* extracts. Acetic carmine staining; magnification: x400 (images from own research).

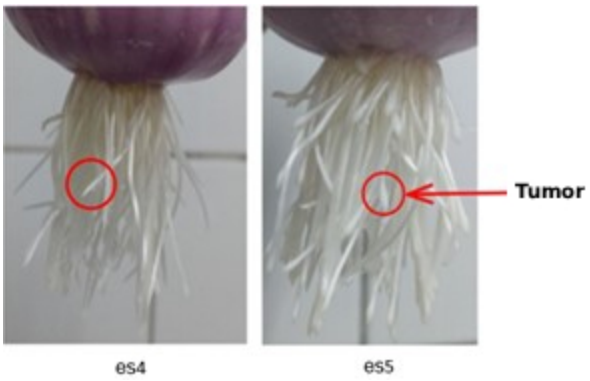

Figure 8

Root tumor formation in *Allium cepa* (es5) (images from own research).