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GENOTOXIC AND CYTOTOXIC EVALUATION OF AQUEOUS EXTRACT OF *ABUTILON PANNOSUM* LEAVES USING *ALLIUM CEPA* ASSAY AND MOLECULAR ANALYSIS IN SAUDI ARABIA

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Abstract

Abutilon pannosum possesses several medicinal benefits, including antioxidants, anti-hepatotoxic, and antidiabetic activities. This study aimed to evaluate the potential genotoxic and cytotoxic effects of aqueous extracts of *Abutilon pannosum* leaves found in Makkah city, Saudi Arabia, using the *Allium cepa* assay and molecular analysis. The Mitotic Index (MI), root growth inhibition (RGI), and chromosomal aberrations (CA) were determined to assess cytotoxicity, while a molecular marker (ISSR-PCR assay) was employed to study genotoxicity. The effective concentration of the aqueous extract (EC50) was determined to be 7.2 mg/mL. Following treatment with *A. pannosum* extracts, a decrease in mitotic index percentage (%MI) was observed along with a concentration-dependent reduction in root tip length. Additionally, chromosomal aberrations such as anaphase bridges, vagrant chromosomes, and C-metaphase were recorded, with the frequency of these aberrations showing a concentration-dependent response to the *A. pannosum* extract. ISSR-PCR markers revealed DNA damage, evidenced by the loss and gain of bands and changes in band intensity. The results of this study concluded that high concentrations of *A. pannosum* extracts exhibit both cytotoxic and genotoxic effects. Therefore, caution should be exercised when using *A. pannosum* for medicinal purposes, and further research is needed to understand its safety profile.

Key words: Medicinal plants, Genotoxicity, Cytotoxicity, Aqueous extract, *Abutilon pannosum, Allium cepa*.

Introduction

Medicinal plants have been a fundamental part of human healthcare systems throughout history, utilized for both preventive and curative purposes across various cultures worldwide (Al-Faifi *et al*., 2017). These plants contain bioactive compounds and can have therapeutic effects on the body (Fan *et al*., 2023). Medicinal plants have effectively played a crucial role during the evolution of human society and utilized for thousands of years as preventive and curative medicines (Singh, 2015; Dar *et al*., 2017). Saudi Arabia is rich in medicinal plants due to the country's rich biodiversity (Rahman *et al*., 2004). With over 80 % of the people in developing countries rely on traditional herbal therapies (Al-Yahya *et al*., 1990; Alanzi *et al*., 2016). Traditional medicines still regularly contribute to the healthcare systems of developing nations. However, despite their widespread use, many medicinal plants have not been thoroughly investigated for their pharmacological properties, leading to potential risks if not properly evaluated (Kufer *et al*., 2010). Comprehensive research into the pharmacological and phytochemical properties of these plants is critical to understanding their potential benefits and adverse effects.

Abutilon (Mill) (Malvaceae) is widely distributed, and six species have been reported in Saudi Arabia: *A. Pannosum*, *A. grandifolium*, *A. fruticosum*, *A. figarianum*, *A. bidentatum* and *A. hirtum,* and these species are commonly found in the Eastern, Southern, and Hijaz regions (Kamal Taia, 2009; Alzahrani *et al*., 2021; Qari *et al*., 2021). Abutilon species contain a variety of chemical substances (Kamal Taia, 2009; Abdel-Rahman Gomaa *et al*., 2016) and have been used in traditional medicine for treating a range of ailments, such as ulcers, gonorrhea, and cough (Aftab & Hakeem, 2021). Leaves, seeds and roots of *A. Pannosum* are used for medical proposes.

Abutilon pannosum belongs to the genus *Abutilon* and is an important high-medicinal value plant (Bano & Deora, 2019)**,** and is rich in secondary metabolites such as phenols, flavonoids, alkaloids, and steroids (Sejalsadhu *et al*., 2016; Aadesariya *et al*., 2019; Qari *et al*., 2021)**.** This plant is used as an antioxidant and anti-hepatotoxic activity (Dave, 2017)**,** antidiabetic, bladder inflammation, lung disorders, diuretic, and lowering pyrexia (Khalil *et al*., 2020), and antimicrobial (Ullah *et al*., 2020)**.** In Saudi Arabia, it is used to cure gonorrhea and diarrhea (Kamal Taia, 2009)**.** Despite its numerous medicinal benefits, certain compounds found in medicinal plants, including those in *A. pannosum*, have been discovered to possess toxic, genotoxic, or carcinogenic properties (Moody *et al*., 1999).

Given the potential risks associated with the medicinal use of plants, it is imperative to conduct thorough evaluations of their genotoxic, cytotoxic, and carcinogenic effects. As part of ongoing research on the safety of medicinal plants used in Saudi Arabia, this study was undertaken to evaluate the cytotoxic and genotoxic effects of the aqueous extract of *A. pannosum* leaves from the Makkah, Saudi Arabia, using molecular and cytogenetic assays on *Allium cepa*. The results of this study showed that treatment of *A. pannosum* extracts showed a decrease in mitotic index percentage (%MI) and a dose dependent decrease in Allium cepa root tip length with increase in the concentration of extracts. Moreover, chromosomal aberrations such as Anaphase bridge, Vagrant chromosome and C- Metaphase were also detected in aqueous extracts and the percentage of chromosomal aberration was increased with higher

concentrations of the extracts. Moreover, Simple Sequence Repeat (ISSR) markers showed DNA damage as observed a loss and gain of bands and changes in band intensity.

Materials and Methods

Sample collection: The leaves of *A. pannosum* samples belonging to the family Malvaceae (Fig. 1) were collected in 2020-2021 from the Makkah in Saudi Arabia at latitude 21°34'30.3"N and longitude 39°46'02.4"E (Fig. 2). The samples were authenticated by a taxonomist in the Department of Biology, Umm Al-Qura University, Saudi Arabia.

Preparation of plant extracts*:* According to the study of (Çelik & Aslantürk, 2010) with modifications in methodology, *A. pannosum* leaves were washed with running tap water followed by three times washing with distilled water (dH_2O) and dried for 72 h at 50˚C in a well-ventilated oven. Dried leaves were ground into a fine powder. Then, 10 mg powder was mixed with 100 ml of dH_2O and boiled for 5 min, left the solution after boiling at 25˚C for 10 min to cool down and then filtered with Whatman filter paper No.1 to remove particles. The solution was diluted with dH_2O, and different range of concentrations were prepared and then used within a Day.

Seed germination of *Allium cepa*: *A. cepa* seeds from the source of Sunshine Seed Co. USA, were used in this study. The seeds were washed twice with dH_2O, and 40 seeds were placed per petri dish containing sterile filter papers. The seeds were grown to germinate in the dark at 25˚C and irrigated with 3 ml dH_2O on the first day and further grown for 5 days and then used for the genotoxicity estimation of aqueous extract at the cellular and molecular level. After culturing the seeds in dH_2O for five days, they were exposed to dH_2O as a control. Then, the seeds were treated with 3ml of different concentrations of *A. pannosum* aqueous extract 1.8, 3.6, 7.2, and 9.0 mg/ml for 24, 48, and 72 hrs. under the same conditions. Root tips were processed, and microscope slides were prepared after 24, 48, and 72 h of treatments (Saghirzadeh *et al*., 2008).

Fig. 1. Picture of *A. pannosum* from the study area in Makkah, Saudi Arabia.

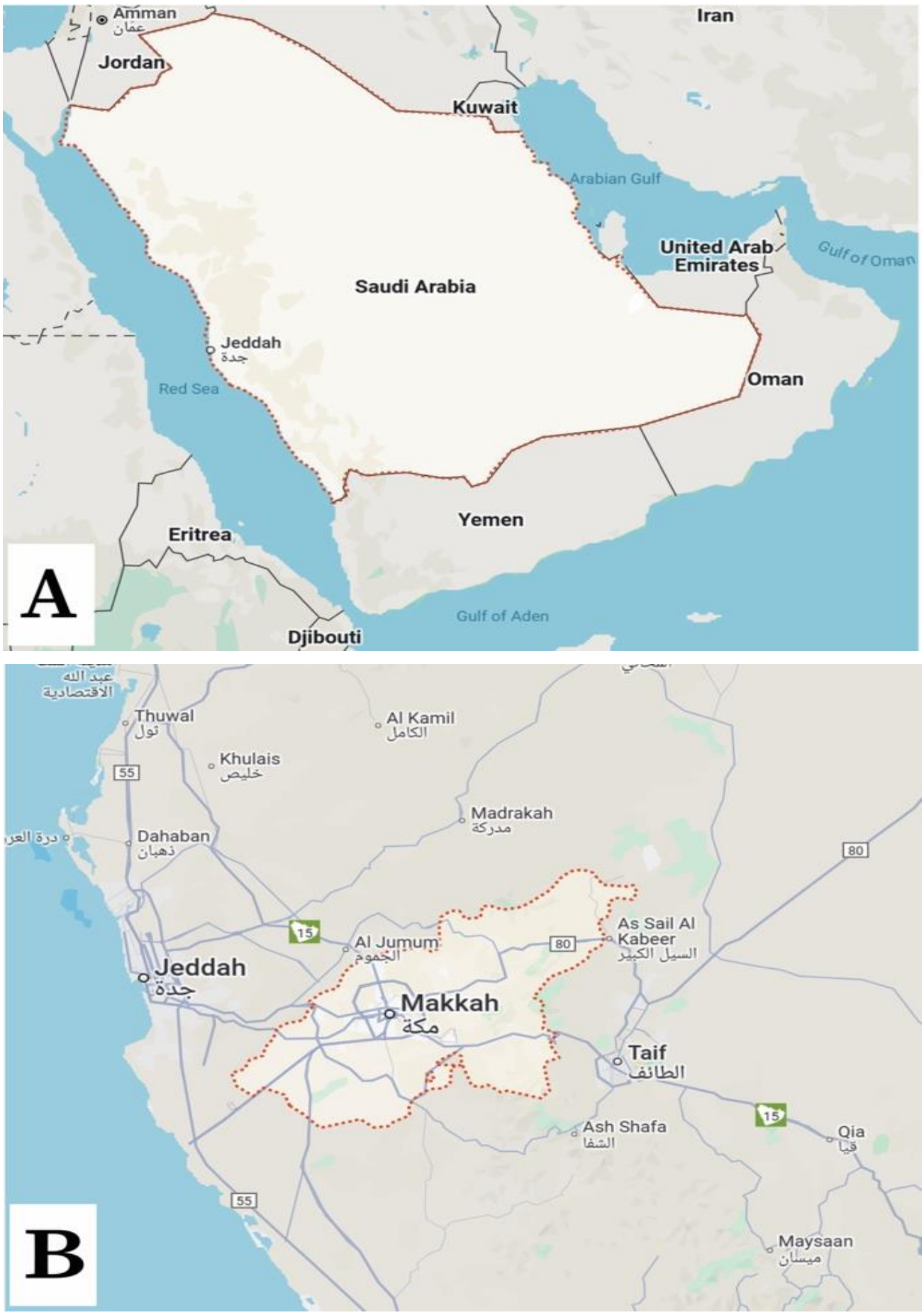

Fig. 2. Site of the samples' collection in the Western region, Makkah, Saudi Arabia (A, B).

Determination of root growth inhibition and EC_{50}: Different concentrations of *A. pannosum* extract were used to calculate the EC_{50} value and assess its *A. cepa* root growth inhibition ability by measuring the root length. Forty seeds of *A. cepa* were washed two times with dH_2O and planted per Petri dish containing a sterile filter paper, then treated with six concentrations of aqueous extract 3, 6, 9, 12, 15, and 18 mg/ml, and dH_2O was used as a control. The seeds were grown for seven days in the dark at 25˚C. The length of roots was measured for each concentration and control. EC_{50} values were determined by interpolation from the germination percentage curve (considering the control as 100%) as described by (Das *et al*., 2021).

Cytogenetic assay

Root length: Roots were measured in mm using a digital ruler after 24, 48, and 72 h of treatment with selected concentrations of *A. pannosum* aqueous extracts and control.

Root tips staining and slides preparation: The slide preparation was done according to (Ping *et al*., 2012) with very slight modification. After pre-treatment, 2 cm root tips were cut off from each concentration and washed five times with dH_2O and placed in a fixative solution (Carnoy's fluid) for 1 h, and hydrolyzed in 1 N HCl at 60-70˚C for 5 min. Root tips were again washed with dH_2O and hydrolyzed for 5 minutes at 60-70˚C in 1 N HCl. After the root tips were washed with dH_2O, a 2 ml root tip was cut and placed on the slide and three drops of aceto-carmine and left for about 2 min then the cover of the slide was carefully placed to avoid air bubbles and dry with tissues. Clear groups of cells on the slides were selected and imaged under a 10x objective lens whereas a 40x lens was used to enlarge the size of cells and view chromosomes.

Chromosomal aberrations and mitotic index: The chromosomal aberrations were reported during this study based on the previous reports (bridge, sticky chromosome, chromosomal fragments, and micronuclei). The mitotic index was calculated according to the following equation (Sidorki, 1984).

$$\text{Mitotic index (MI)} = \frac{\text{Number of dividing cells}}{\text{Total number of cell}} \times 100$$

Molecular assay: Standard molecular tests were used to assess the genotoxicity effects of total DNA samples isolated from *A. cepa* roots after exposure 72 h to different concentrations of *A. pannosum* aqueous extract and control. After DNA extraction was achieved, PCR ISSR test and gel electrophoresis were carried out for further molecular analysis.

DNA extraction and purity/concentration measurement: The CTAB technique was followed to extract *A. cepa* DNA according to (Allen *et al*., 2006) with some modifications. DNA samples were diluted with water according to the Nanodrop manual (Thermo Fisher Scientific) to measure DNA purity and concentration. 1µl of nuclease-free water was placed in a Nanodrop device without bubbles and the blank option was selected. Then, after cleaning with tissue, 1µl from each DNA sample was added to Nanodrop, and then measured and the results documented.

Extracted DNA from the samples treated with the highest concentration of *A. pannosum* extracts were diluted to 50 ng/µl having an absorbance of 1.80 ± 0.08 at a wavelength of 260/280, displayed details results of DNA concentrations (before and after dilution) and DNA purity shown in (Table 1). Then, the isolated DNA was used for PCR analysis with ISSR primers.

ISSR-PCR Marker: To assess the potential genotoxic of *A. pannosum,* molecular markers were used Inter Simple Sequence Repeat (ISSR-PCR) for polymorphism and to evaluate the genotoxic potential of the aqueous extract. After extracting DNA from the *A. cepa* root, four ISSR primers were used as explained in (Table 2) and in (Eröz Poyraz *et al*., 2018; Poyraz, 2022).

Table 1. DNA (*A. cepa*) concentrations and purity in samples treated with *A. pannosum*.

Exposure times h	Concentration (mg/ml)	DNA concentration before dilution, ng/µl	Dilution ratio	DNA concentration after dilution, ng/µl	Absorbance at 260/280
	control	1210	1:25	48.40	1.80
	1.80	1830	1:7	55.14	1.88
72	3.60	810	1:15	54.0	1.86
	7.20	954	1:20	47.70	1.78
	9.00	1084	1:20	54.20	1.88

Table 2. ISSR Primers used in PCR amplification.

Primer name	5'-3' sequence	Melting T°
ISSR 418	CTCTCTCTCTCTCTCTTG	42
ISSR HB12	CAGCAGCAGGC	42
ISSR UBC-811	GAGAGAGAGAGAGAGAC	42
ISSR MAO	CTCCTCCTCCTCRC	42

Polymerase chain reaction (PCR) amplification: Four ISSR Primers were used for the PCR amplification of DNA isolated from *A. cepa* roots. The annealing temperature was optimized for each primer to 42˚C. ISSR-PCR kit and solutions were purchased from Sigma-Aldrich (ReadyMix™ Taq PCR Reaction Mix Cat. No P6400), and the primers were ordered from Macrogen, Sout Korea. The DNA ladder (MoleculeOn) of 1000 bp was used having 10 equal bands of 100 bp each. The five samples were used in total including four samples of different concentrations of aqueous extraction and one sample representing the control. Merck PCR kit had a master mix containing $MgCl_2$, dNTP, assay buffer, and Taq polymerase. PCR

program consisted of 30 repeated cycles followed by a final extension for 7 min at 72˚C. The last step was the holding of samples at 4˚C.

Gel electrophoresis: After (PCR) amplification, gel electrophoresis was performed as described in (Shaaban, 2022). The sample solution was loaded into the gel wells with the DNA ladder in the first well of the gel and the tank was filled with 1X TBE buffer, and the gel was run at 100V for 1 hour and 20 min. Then, the gel was placed in a Gel Documentation system, and the bands were observed, imaged and compared under UV light (Haglund, 2022).

Data Analysis

The data were statistically investigated using analysis of variance (ANOVA), which demonstrated significant differences ($p<0.05$) in the mean root lengths of *A. cepa* against various concentrations of *A. pannosum* leaves aqueous. Also, Quantity One analysis software version (4.6.2) was used to analyze gel electrophoresis bands and generate phylogenetic dendrograms.

Results

Determination of EC_{50} and root growth inhibition: The results of leaves' *A. pannosum* effects on the root growth of A. cepa are shown in (Fig. 3). The concentrations of *A. pannosum* leaves aqueous extract caused a 50% reduction in root growth of A. cepa (EC_{50}) in comparison to the control. These figures depict a linear decrease in the root length with the rising concentrations of aqueous extract of *A. pannosum* leaves. The two parameters had a well-fitting coefficient of determination for the aqueous ($R^2 = 0.90$) extract (Fig. 3). The median effective concentration values (EC_{50}) of the aqueous extract of *A. pannosum* leaves were calculated as 7.2 mg/ml 0.50%. The EC_{50} value is crucial for selecting test concentrations to conduct genotoxicity assays.

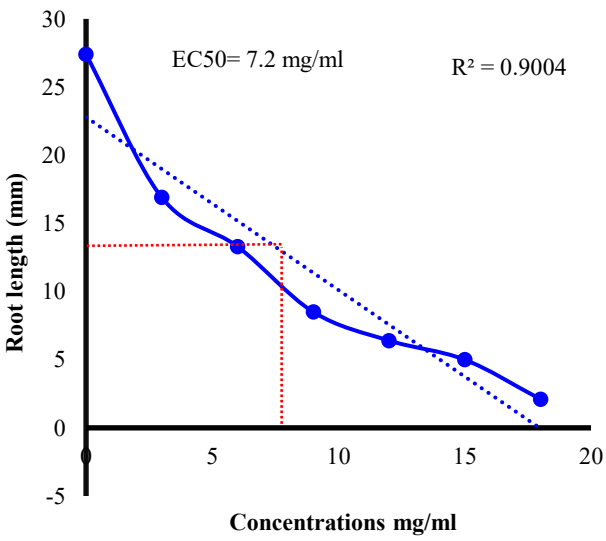

Fig. 3. EC_{50} value was responsible for 50% root growth inhibition calculated from the relationship of different concentrations of *A. pannosum* leaves aqueous extract.

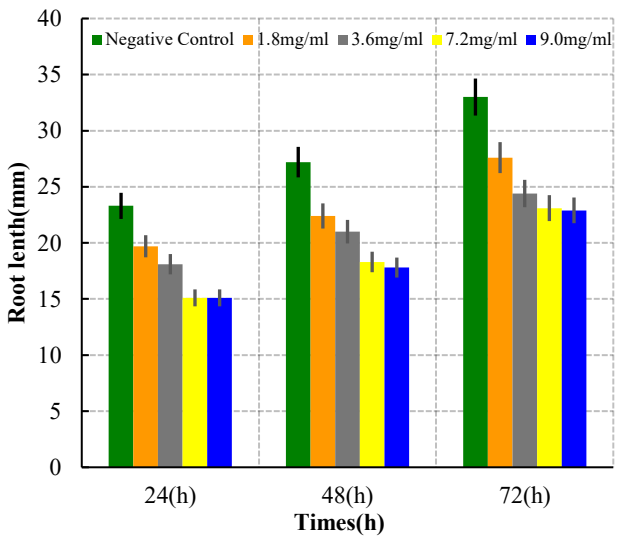

Fig. 4. Effect of different concentrations of *A. pannosum* aqueous extract on the root length of Allium cepa over 24, 48, and 72 hours. Error bars represent the standard deviation of the mean. Asterisks denote statistically significant differences compared to the control ($p<0.05$).

Table 3. Cytogenetic analysis of *A. cepa* root tips treated by different concentrations of aqueous extract for different exposure times.

Exposure time (h)	Concentration (mg/ml)	Total cells counted ± SD	Total cell division ± SD	Mitotic index % ± SD	Chromosome aberration % ± SD
24	control	1003 ± 2.52*	94 ± 4.16*	9.40 ± 0.43*	0.24 ± 0.38*
	1.8	1005 ± 5.57*	91 ± 3.06*	9.02 ± 0.33*	1.96 ± 0.51*
	3.6	1004 ± 4.58*	83 ± 5.69*	8.30 ± 0.55*	4.27 ± 1.26*
	7.200	1008 ± 3.46*	77 ± 8.50*	7.67 ± 0.87*	4.74 ± 0.88*
	9	1004 ± 4.04*	69 ± 9.50*	6.91 ± 0.97*	6.41 ± 1.02*
48	control	1009 ± 2.00*	86 ± 5.00*	8.52 ± 0.51*	0.90 ± 0.69*
	1.8	1009 ± 1.53*	93 ± 5.69*	9.19 ± 0.58*	1.68 ± 0.51*
	3.6	1007 ± 7.64*	89 ± 3.00*	8.84 ± 0.23*	2.87 ± 0.69*
	7.200	1009 ± 10.26*	76± 11.02*	7.56 ± 1.02*	4.66 ± 0.69*
	9	1004 ± 4.16*	70 ± 8.50*	6.94 ± 0.84*	6.22 ± 0.88*
72	control	1005 ± 5.13*	92 ± 3.46*	9.15 ± 0.31*	0.85 ± 0.51*
	1.8	1006 ± 4.51*	95 ± 6.11*	9.41 ± 0.64*	2.11 ± 0.88*
	3.6	1004 ± 4.51*	89 ± 1.53*	8.89 ± 0.15*	4.23 ± 0.51*
	7.200	1007 ± 2.52*	70 ± 9.50*	6.98 ± 0.96*	6.79 ± 0.69*
	9	1006 ± 4.93*	63 ± 8.33*	6.30 ± 0.85*	8.60 ± 0.51*

Root growth measurement after treatment by *A. pannosum* extracts: The average root lengths of *A. cepa* treated with varying concentrations of the aqueous extract were measured at 24, 48, and 72 hours (Fig. 4). A concentration- and time-dependent decrease in root length was observed. After 72 hours of exposure to the highest concentration (9.0 mg/mL), the average root length was reduced to 17.85 mm, compared to 31.8 mm in the control group.

Statistical analysis using Pearson's correlation revealed a significant negative correlation between extract concentration and root growth ($r = -0.90$, $p<0.05$), confirming that root growth inhibition was significantly dependent on the concentration of the aqueous extract. Lower concentrations (1.8 mg/mL) resulted in higher growth rates compared to higher concentrations (9.0 mg/mL), indicating that the extract's inhibitory effect intensifies with increasing concentration. Significant differences in root growth were also observed between different concentrations at each time point ($p<0.05$).

Cytogenetic effects on *Allium cepa* meristematic cells: The cytogenetic effects of different treatments of *A. pannosum* aqueous extract and control, at various exposure periods of 24, 48, and 72 h were studied, and 3000 cells were counted per treatment (Table 3 and Fig. 5). The *A. cepa* root tip assay revealed that after 48 h of treatment, the mitotic index decreased to 6.94% with the highest concentration (9.0 mg/ml) in comparison with 8.52% of the control (Table 3). The highest concentration (9 mg/ml) of *A. pannosum* extract increased the percentage of chromosomal aberration to 8.60% as compared to 0.85% in the control (Table 3 and Fig. 5). Moreover, chromosomal aberration rates also varied with different concentrations of aqueous extract and exposure periods. The rate of chromosomal aberration increased at higher concentrations and longer exposure periods. After 24 h, 4.89% chromosomal aberration was noted at the highest concentration of 1.437 mg/ml (Table 3 and Fig. 5). The observed aberrations suggest genotoxic stress induced by the extract, which impacts the integrity and accurate segregation of chromosomes during cell division.

Molecular analysis of extracted DNA from treated *A. cepa* roots using ISSR markers: Molecular analysis utilizing ISSR markers was conducted to further evaluate the genotoxic potential of the aqueous extract. Data is presented in Fig. 6A and B showed that the four primers (ISSR-418, ISSR-HB12, ISSR-UBS811, and ISSR-MAO) gave a total of more than 9 genetic bands ranging from 510 to 1152 bp through one primer with each of the different treatments. Different polymorphic bands and genotypes separated from each treatment compared to the control treatment were detected at 1.8, 3.6, 7.2, and 9.0 mg/ml of *A. pannosum* for 72 h. The value of the polymorphic rate for the four primers was 75%, 60%, 20%, and 62.5% respectively. In all cases, polymorphisms were due to the loss and/or gain of amplified bands in the treated samples compared with the control.

Also, (Fig. 7) show the phylogenetic dendrogram of four Primers as a marker to show the relationship between treatments of different concentrations of *A. pannosum* aqueous extract compared to control as shown by UPGMA analysis. The result showed a big variance of all primers between the control and concentration 9.0 mg/ml (Fig. 7). The results showed that some clades have low genetic variance across all primers, with varying distances from each other. This suggests a convergence in evolutionary relationships between the control group and the treatments, depending on concentration differences. Moreover, clades with high concentrations, such as 7.2 and 9.0 mg/ml, exhibited differences based on the primers used, positioning them farther from the control clade (Dendrogram2). In contrast, some of the low concentrations, such as 1.8 and 3.6 mg/ml, moved closer to the control clade (Dendrogram1).

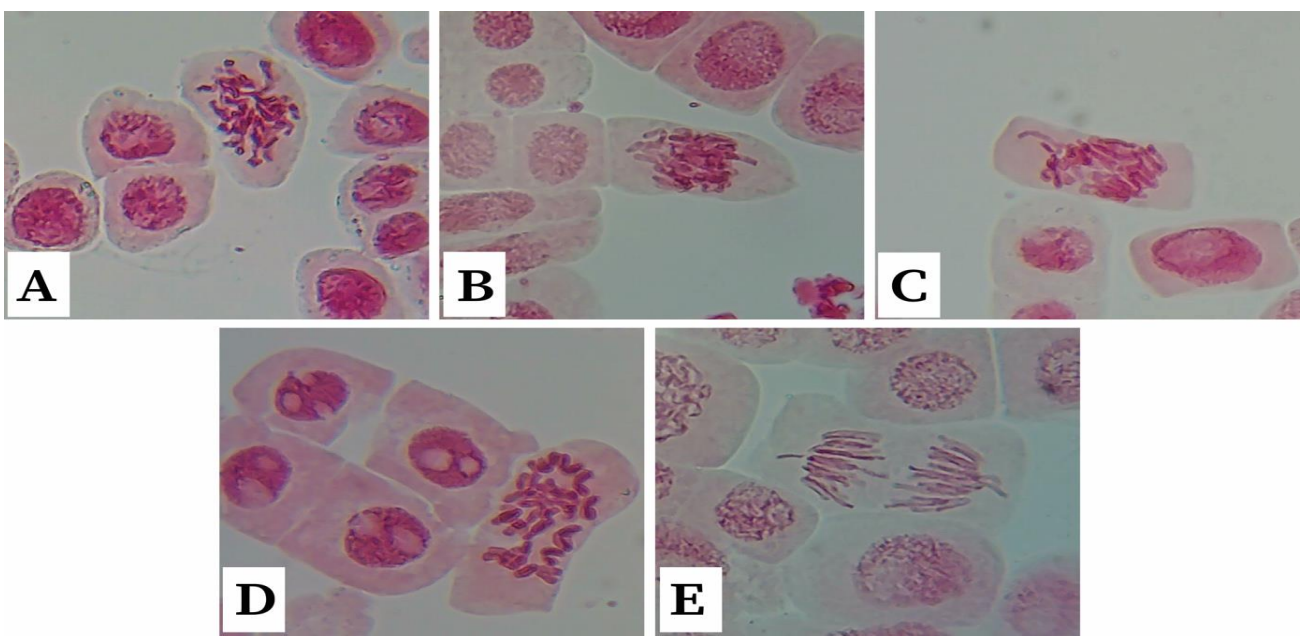

Fig. 5. Different types of *A. cepa* meristematic cells showed chromosomal aberration after being treated with *A. pannosum* leaves aqueous extract for 24, 48, and 72 h. **(A)** Disrupted metaphase after 48 h at 9.0mg/ml **(B)** Chromosome fragments in metaphase after 48 h at 7.2mg/ml **(C)** Chromosome fragments in anaphase after 24 h at 9.0mg/ml **(D)** C-Metaphase after 72 h at 9.0mg/ml **(E)** Vagrant chromosome in anaphase after 48 h at 7.2mg/ml.

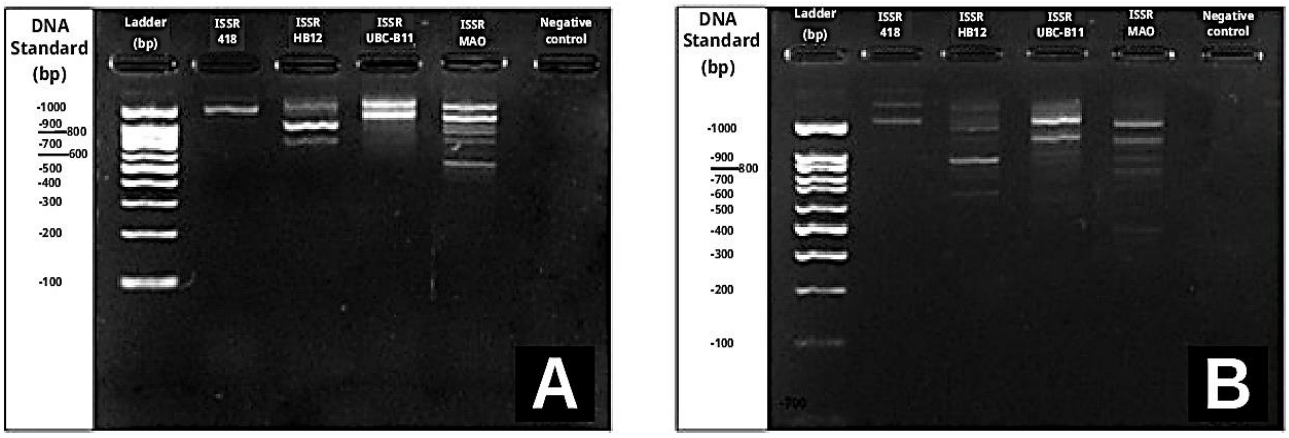

Fig. 6. Gel electrophoresis bands of amplified PCR products (A): for control after 72 h. (B): for the highest aqueous extract concentration of 9.0 mg/ml. Obtained with ISSR primers (ISSR 418, ISSR HB12, ISSR UBC-811, and ISSR MAO.

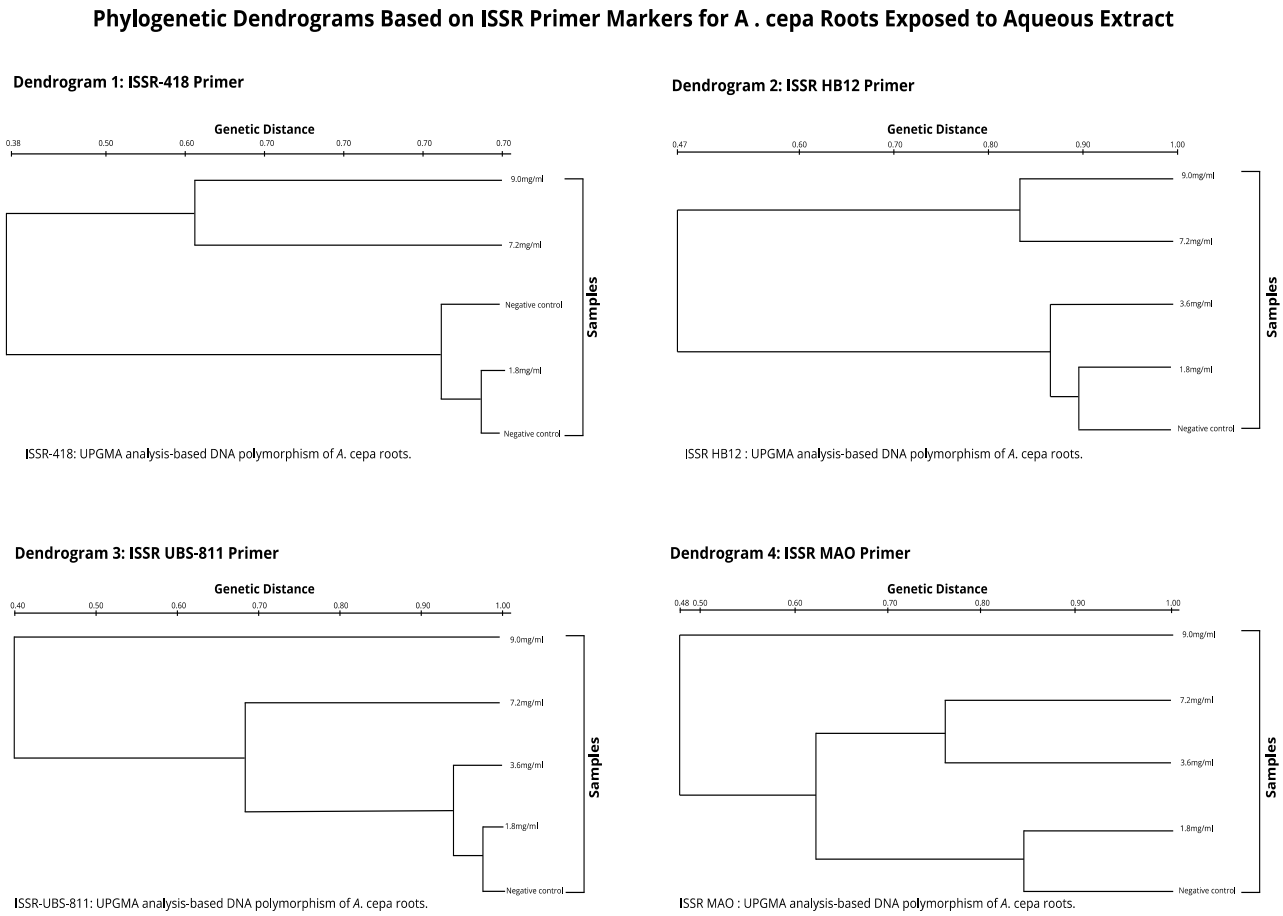

Fig. 7. Phylogenetic dendrograms were constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) to illustrate the genetic relationships among the *A. pannosum* treatments. **Dendrogram 1:** Generated using the ISSR418 primer, this dendrogram demonstrates the clustering of treatments based on genetic similarity. **Dendrogram 2:** Utilizing the ISSR-HB12 primer, this dendrogram indicates the genetic distances between treatments. **Dendrogram 3:** Created with the ISSR-UBC811 primer, this dendrogram highlights unique genetic profiles among the treatments. **Dendrogram 4:** Employing the ISSR-MAO primer, this dendrogram illustrates significant genomic changes at higher treatment concentrations.

Discussion

This study investigated the potential genotoxic and cytotoxic effects of the aqueous extract of *A. pannosum* leaf on A. Cepa root cells at both cellular and molecular levels. Our data showed that different concentrations of *A. pannosum* leaf aqueous extract caused a dose-dependent decrease *in A. cepa* root length compared to the control. The EC_{50} value, selected at 7.2 mg/ml, was crucial for determining appropriate test concentrations for subsequent cytotoxicity and genotoxicity assays (Özkara *et al*., 2015; Akwu *et al*., 2019; Das *et al*., 2021). The consistent observation of shorter root length in all treated groups relative to the control indicates that the extracts

have significant cytotoxic effects on meristematic cells, thereby inhibiting A. Cepa root growth (Alabi *et al*., 2022). The dose-dependent inhibition of *A. cep*a root growth serves as a primary indictor of the extract's cytotoxic potential. This result aligns with several studies demonstrating that various phytochemicals in plant extracts can impair root elongation by interfering with important cellular processes such as cell expansion, cell division, or nutrient uptake in meristematic tissues. Consistent with this, the mitotic index (MI) is a key indicator of cytotoxicity (Chukwujekwu & Van Staden, 2014), which exhibited a significant decrease with increasing concentrations of the aqueous extracts. Specifically, at 9 mg/ml of *A. pannosum* after 72 hours, a notable reduction in MI was observed, indicating an inhibition of cell division activity and possibly a disruption in the ratio of mitotic phases. This reduction in MI directly correlated with the observed root growth inhibition in *A. cepa*. A decrease in mitotic index suggests a cytostatic effect, where the extract either arrests cells in interphase, delays their entry into mitosis, or induces cell death, consequently reducing the number of dividing cells essential for root elongation. This cytostatic action is a common mechanism by which natural extracts with cytotoxic properties exert their effects, often by disrupting cell cycles or interfering with DNA synthesis and repair. Our findings suggest that *A. pannosum* aqueous extracts likely block cellular progression thorough one or more cell cycle phases, leading to the observed decrease in mitotic index. These results are in line with previous studies showing that various plant aqueous extracts can cause mitotic inhibition and cell cycle disruptions (Akinboro & Bakare, 2007; Çelik & Aslantürk, 2010; Ihegboro *et al*., 2020; Ei-Ghamery *et al*., 2000; Fatemeh & Khosro, 2012).

Extending beyond general cytotoxicity, our investigation revealed the genotoxic effects of *A. pannosum* extract on *A. cepa* meristematic cells. A significant, concentration- and time-dependent increase in chromosomal aberration frequency was observed, serving as a compelling indicator of DNA damage. The diverse array of chromosomal aberrations documented, including disrupted metaphase, chromosome fragments, C-metaphase, and vagrant chromosomes, provides important insights into the underlying genotoxic mechanisms. Notably, the highest rate of chromosomal aberrations was recorded at a concentration of 9 mg/ml. Chromosome fragments directly signify clastogenic activity, implying that components within the extract induce breaks in the DNA molecule of *A. cepa* meristematic cells. The presence of C-metaphase and disrupted metaphase strongly suggests an aneugenic effect, wherein the extract interferes with the mitotic spindle apparatus, leading to aberrations in chromosome alignment and segregation(Akinboro & Bakare, 2007). Moreover, vagrant chromosomes highlight defects in spindle formation, resulting in chromosomes failing to properly integrate into the daughter nuclei during anaphase. Collectively, these observed alterations indicate that the *A. pannosum* extract contains compounds capable of inducing various chromosomal abnormalities, thereby potentially compromising genomic integrity and cell viability. Such chromosomal defects, including vagrant chromosomes, have also been reported in studies involving other medicinal plant extracts (Alabi *et al*., 2022; Akinboro & Bakare, 2007).

The molecular analysis utilizing ISSR markers provided robust and complementary evidence of the genotoxic potential of *A. pannosum* extract, thereby corroborating the preceding cytogenetic findings. The use of four distinct ISSR primers (ISSR-418, ISSR-HB12, ISSR-UBC811, and ISSR-MAO) revealed substantial changes in the genomic DNA sequence of treated samples compared to the control. These changes were manifested as the detection of novel bands (gain) and the disappearance of pre-existing bands (loss) within the amplification profiles. This observed polymorphism, particularly pronounced at higher concentrations of *A. pannosum* aqueous extracts, signifies underlying genomic alterations. Such alterations can encompass point mutations, insertions, deletions, or rearrangements that affect the primer binding sites or the intervening DNA sequences. The differential polymorphic rates observed across the four primers (75% for ISSR-418, 60% for ISSR-HB12, 20% for ISSR-UBC811, and 62.5% for ISSR-MAO) show that the genotoxic effects are not uniformly distributed throughout the genome but may rather be sequence-specific or preferentially target certain genomic regions. In addition, the gain or loss of bands can be directly attributed to DNA strand breaks or mutations occurring within the primer annealing regions, which subsequently change the DNA fingerprint (Enan, 2007; Bernardes *et al*., 2015). Consequently, these observed modifications in band patterns serve as clear indicators of DNA damage, collectively exhibiting a state of genomic instability within the organism. Such instability is frequently linked to mutations that disrupt the cellular processes involved in DNA replication, repair, and cell division (Fenech, 2005; Bernardes *et al*., 2015).

Furthermore, similar findings regarding genotoxicity were reported by Andrade-Vieira *et al*., (2018), who analyzed the effects of Spent Potliner (SPL) and its derivatives on *A. cepa* and *Lactuca sativa*. Their study also showed mutagenicity via ISSR markers, evidenced by changes in bands between test and control samples and a distinct clustering of control samples in the phylogenetic dendrogram compared to treated samples. Similarly, the phylogenetic dendrograms were constructed from our ISSR-PCR bands data visually confirm the genetic divergence induced by the *A. pannosum* extracts. The clustering patterns clearly demonstrate significant genetic variance between the control and higher concentration treatments (e.g., 9.0 mg/ml), positioning them farther apart in the dendrogram. Conversely, lower concentrations (1.8 and 3.6 mg/ml) show closer genetic relationships to the control, reflecting a less pronounced impact on the genome of *A. cepa* plant. This dose-dependent genetic divergence, detected at the molecular level, strongly reinforces the dose-dependent effects observed in our root growth and cytogenetic assays.

Conclusion

Abutilon pannosum is known for its medicinal benefits, including anti-hepatotoxic, antioxidant, and antidiabetic activities. However, our comprehensive analysis using root growth inhibition, cytogenetic tests, and ISSR markers revealed significant cytotoxic and genotoxic effects of its aqueous leaf extract on *Allium cepa* at the tested concentrations. The consistent dose- and time-dependent responses across all three tests highlight the presence of bioactive compounds in the extract that can interfere with normal cellular processes and compromise genomic integrity. While plant extracts are often explored for their therapeutic properties, these findings underscore the critical importance of thoroughly evaluating their safety, particularly concerning potential genotoxicity. Medicinal plant extracts must be used with extra caution due to their possible toxic side effects at the molecular level when used inappropriately. Further studies are needed to fully evaluate other biological activities of *A. pannosum* and to ensure its safety for human therapeutic use. Future research should focus on isolating and characterizing the specific phytochemicals responsible for the observed cytotoxic and genotoxic effects in the *A. pannosum* aqueous extract. Detailed mechanistic studies are also warranted to elucidate the precise molecular pathways through which these compounds induce mitotic inhibition, chromosomal aberrations, and DNA polymorphisms.

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