Journal of Umm Al-Qura University for Applied Sciences
https://doi.org/10.1007/s43994-025-00248-z

ORIGINAL ARTICLE

Genotoxic analysis of aqueous extract of *Aerva javanica* leaves on *Allium cepa*: cytogenetic and molecular assessment

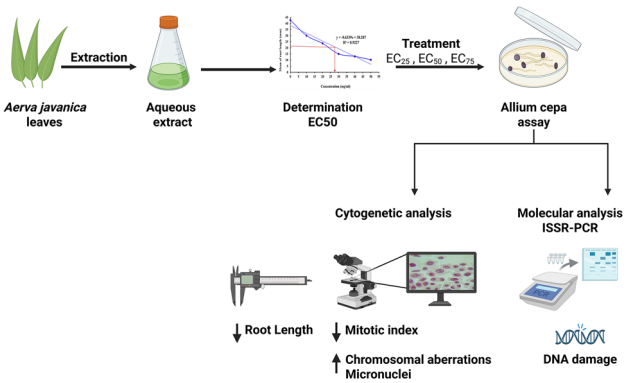
Maryam Mousa Alzahrani[1] · **Abdulmajeed F. Alrefaei**[1] · **Hassan O. Shaikhaldein**[2]

Received: 14 March 2025 / Accepted: 1 June 2025
© The Author(s) 2025

Abstract

Aerva javanica is a significant medicinal plant with a wide geographic distribution, prevalent in many countries. The plant is employed in traditional healing practices to address a range of illnesses. This study aimed to evaluate the potential genotoxicity of aqueous *A. javanica* leaf extract on *Allium cepa* roots using cytogenetic and molecular assays. The effective concentration (EC_{50}) of the water-based leaf extract was determined to be 26.7 mg/mL. Based on this value, three concentrations were selected to evaluate genotoxicity: below the EC_{50} (13.35 mg/mL), the EC_{50} itself (26.7 mg/mL), and above the EC_{50} (40.05 mg/mL). To establish a baseline for comparison, distilled water was utilized as a control. The inhibition of root growth was assessed, and cytotoxicity was determined by the mitotic index (MI). In addition, genotoxicity was further analyzed through the assessment of chromosomal aberrations (CA), micronuclei (MN), and Inter-Simple Sequence Repeat (ISSR) Polymerase Chain Reaction (PCR) markers as a molecular assay. Increasing the amount of *A. javanica* leaf extract resulted in a progressive shortening of root length. Additionally, at higher concentrations MI percentage decreased, indicating cytotoxicity, while CA and MN frequencies increased, suggesting genotoxicity. Furthermore, DNA damage was confirmed through ISSR-PCR marker analysis. These findings indicate that higher concentrations of *A. javanica* leaf extract induce genotoxic effects, highlighting the need for studies of its human use in therapeutic applications.

Graphical abstract



Keywords Medicinal plant · Cytotoxicity · Genotoxicity · *Aerva javanica* · *Allium cepa* assay · ISSR markers

✉ Abdulmajeed F. Alrefaei
afrefaei@uqu.edu.sa

[1] Department of Biology, Jamoum University College, Umm Al-Qura University, 21955 Makkah, Saudi Arabia

[2] Botany and Microbiology Department, College of Science, King Saud University, P. O. BOX 2455, 11451 Riyadh, Saudi Arabia

1 Introduction

Medicinal plant extracts are prescribed medications in many developed countries [1]. Meanwhile, the World Health Organization states that a significant majority—80%—of people across the globe utilize traditional medicine for

Published online: 30 July 2025

at least some primary health needs [2]. In Saudi Arabia, medicinal plants are used to treat diverse human and animal illnesses [3]. In a survey on herbal medicine use in Saudi Arabia, 88.4% of respondents stated they utilize herbal remedies, while 84.3% expressed interest in traditional recipes. Furthermore, 88.7% used herbal medicine for therapeutic purposes, with a reported effectiveness rate of 61.2% [4]. Among the medicinal plants used in Saudi Arabia, *Aerva javanica*, a perennial shrub belonging to the Amaranthaceae family, possesses considerable therapeutic and market value. According to Ayurvedic tradition, this plant stands out as a primary resource for natural remedies [5].

Due to their ethnopharmacological properties, extracts from these plants are highly valued for medicinal and therapeutic applications [6]. Aerial parts of *A. javanica* are boiled into a decoction to produce a diuretic traditional remedy for hypertension, rheumatism, headaches, swelling, and kidney stones [7]. Root decoctions are used to alleviate toothache, headache, and diarrhea, while pastes are used to clear facial acne. The plant also serves as a laxative and emetic for camels and horses. Moreover, the extract of aerial *A. javanica* portions is used to treat dysentery, gonorrhea, hyperglycemia, and dermatological diseases [8]. A leaf paste is applied externally to heal wounds, reduce inflammation, and treat granulation tissue [9]. Whole-plant extracts exhibit significant antimicrobial activity and serve as disinfectants in alternative medicine [10]. They also possess significant antioxidant activity [11–13], while certain extracts exhibit antidiarrheal activity [14]. Alongside these established uses, potential anticancer properties of *A. javanica* have recently been identified [15]. It also possesses medicinal properties for treating rheumatism and renal diseases, and for relieving toothaches [16].

Analysis of the chemical composition of *A. javanica* has shown the presence of glycosides, flavonoids, saponins, and phenols [17, 18]. While the popularity of herbal products is often linked to their perception as safer and more effective than allopathic drugs, this assumption is erroneous and potentially detrimental. Therefore, toxicological investigations of herbal remedies are essential [19]. In this context, it has been reported that some traditional treatments may exhibit genotoxic properties [20, 21]. While several studies have evaluated the biological effects of *A. javanica* extracts, the current study demonstrates the first comprehensive assessment of the genotoxic potential of aqueous *A. javanica* leaf extract. A combination of cytogenetic and molecular assays (ISSR-PCR) is used to analyze DNA damage and chromosomal abnormalities induced in *Allium cepa* meristem cells.

The *A. cepa* test model is a well-established tool for determining the toxicological potential of plant extracts [22], serving as a powerful bioindicator of toxic effects. The ISSR molecular marker has also emerged as a reliable technique for evaluating DNA damage [23]. Thus, the present investigation aims to investigate the genotoxicity of an aqueous extract of *A. javanica* leaves on *A. cepa* meristem cells using cytogenetic and molecular assays.

2 Methods and materials

2.1 Samples collection

Specimens of *A. javanica* were gathered in May 2023 from Umm Al-Qura University, Makkah, Saudi Arabia (*coordinates:* 21.330189322777255 N and 39.95250641319426 E). The samples were identified and classified by a scientist at the Department of Biology.

2.2 Formation of aqueous extract of *A. javanica* leaves

Fresh *A. javanica* leaves were thoroughly rinsed multiple times with tap water and then three times with distilled water. The leaves were then dried in a hot-air oven (Binder, FD56) at 40 °C for 72 h. Dried samples were ground into a fine powder with an electric blender and kept in a sealed container at 4 °C, as previously described [24]. An aqueous extract of *A. javanica* leaves was prepared using a modification of a previously described protocol [25]. A 5-g sample of desiccated *A. javanica* leaf powder was measured and boiled in 100 mL of distilled water for 5 min. The mixture was brought to room temperature and filtered using Whatman No. 1 filter paper. Various concentrations were prepared by dilution and stored at 4 °C. Preparations were used within 24 h for each experiment. Figure 1 illustrates the preparation of the aqueous extract of *A. javanica* leaves.

2.3 Identification of EC_{50}

The EC_{50} value was determined using several concentrations of aqueous *A. javanica* leaf extract by a modified form of a technique described previously [26]. *A. cepa* seeds were washed twice with distilled water and subsequently dried at 25 °C. Sterile filter paper was positioned in each petri dish (11 cm diameter), and twenty seeds were germinated with 4 mL of distilled water for four days at 25 ± 1 °C in the dark. Twenty germinated seeds with approximately equal root lengths were selected and positioned on filter paper in separate Petri dishes. The roots were treated with 4 mL of five concentrations of the aqueous *A. javanica* extract (10, 20, 30, 40, and 50 mg/mL), or distilled water as a control. The Petri dishes were sealed and kept at 25 ± 1 °C for three days. Subsequently, the lengths of eighty roots for each concentration and the control were measured in millimeters (mm), and the average root length was calculated for each.

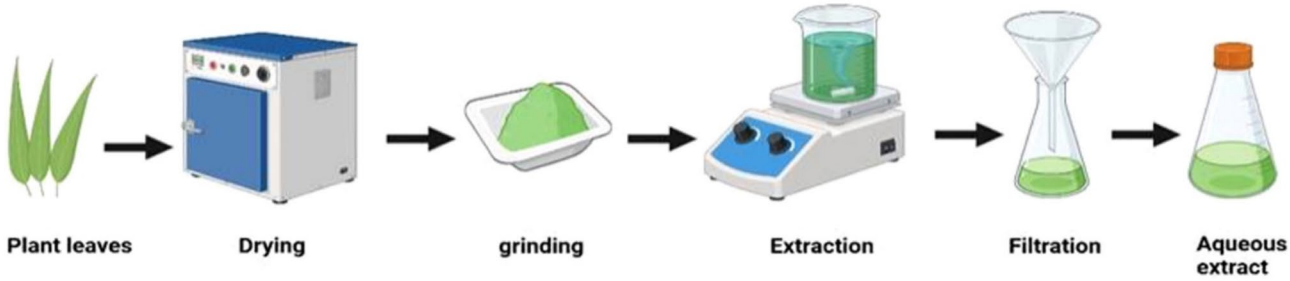


Fig. 1 Preparation steps of aqueous extract of A. javanica leaves

A root length growth curve was plotted in comparison to the control, and the EC_{50} value—representing a 50% inhibition of root growth compared to the untreated samples—was determined.

2.4 Cytogenetic analysis

2.4.1 Assay of *Allium cepa*

A. cepa seeds (Emerald Seed Inc., USA) were rinsed with 70% ethanol for 5 s, followed by five washes with distilled water, and then dried at 25 °C. Thirty seeds were positioned in each Petri dish containing sterile filter paper and 4 mL of distilled water. Multiple Petri dishes were prepared and incubated in the dark at 25 ± 1 °C for four days until germination. Subsequently, twenty germinated *A. cepa* seeds with approximately 6 mm root lengths were transferred to separate Petri dishes with sterile filter paper. These roots were treated with 4 mL of three concentrations of aqueous *A. javanica* leaf extract, corresponding to EC_{25}, EC_{50}, and EC_{75} (13.35, 26.7, and 40.05 mg/mL, respectively), alongside a distilled water control. The dishes were subjected to dark incubation at 25 ± 1 °C for 24, 48, and 72 h, with all conditions remaining constant. Root length was measured in millimeters (mm) using a digital ruler at each time point for each treatment. Additionally, *A. cepa* root tips were cut to 2 cm, washed five times with distilled water, and then subjected to fixation in Carnoy's solution (glacial acetic acid and ethanol in a 1:3 mixture) for 90 min, as previously described [27]. Subsequently, the root tips were rinsed five times with distilled water and preserved in ethanol (70%) at 4 °C until slide mounting. Root tips that were contaminated, failed to germinate properly, or appeared physically damaged at any stage were excluded to assure the accuracy and consistency of the results.

2.4.2 Slide preparation

Root tips were cleansed with distilled water before being hydrolyzed in 1 N HCl for 3 min at 60–65 °C. The 1 N HCl solution was made by combining 8.3 mL of HCl and 100 mL of distilled water [28]. Following hydrolysis, root tips underwent five successive rinses with distilled water before being cut (1–2 mm) and positioned on a slide. Drops of acetocarmine stain [29], stored in a dark bottle at 4 °C and used within one day of production, were added to slides and allowed to incubate for approximately 15 min. After staining, a coverslip was carefully placed on the slide and sealed with clear nail polish [30]. Three slides were prepared per treatment for each exposure time, with two root tips placed on each slide. The slides were analyzed through a light microscope (LABOMED, LB-210) with a 40× objective lens to count cells and capture images. A total of 3000 cells were studied for each treatment at each exposure time [26].

2.4.3 Mitotic index (MI) and chromosomal aberrations (CA) analysis

The cells in each treatment were assessed to evaluate the mitotic index (MI) as well as chromosomal aberrations (CA). Scored CA included chromosomal bridges, chromosomal fragments, vagrant chromosomes, chromosomal disturbances, micronuclei (MN), C-metaphase, and sticky chromosomes. The MI was calculated according to [31], as follows:

$$\mathrm{MI} = \frac{\text{dividing cells/number}}{\text{the complete cell amount}} \times 100$$

2.5 Molecular analysis

Molecular analysis was performed to study the genotoxic effects of 72-h exposure to aqueous *A. javanica* leaf extract on DNA samples purified from the roots of *A. cepa*. Figure 2 illustrates the procedures of molecular analysis.

2.5.1 DNA extraction

DNA samples were isolated from *A. cepa* roots after 72 h of treatment with several concentrations of the aqueous *A.*

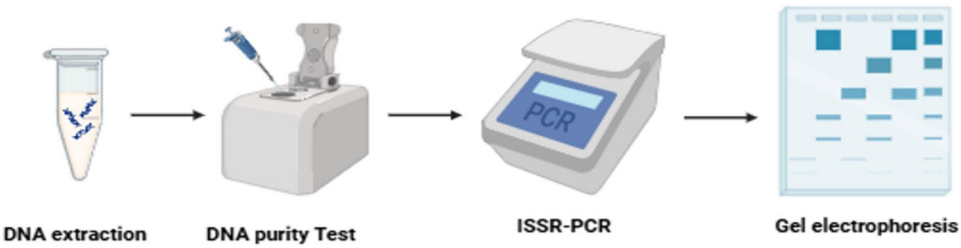


Fig. 2 Steps of molecular analysis using ISSR markers

Table 1 ISSR Primers used in the study

Primer name	5′–3′ sequence	Annealing temperature °C	Application in literature	References
ISSR 418	CTCTCTCTCTCTCTCTTG	51	Genotoxicity and genetic diversity in *A. cepa*	[34, 35]
ISSR HB12	CAGCAGCAGGC	36		
ISSR UBC-811	GAGAGAGAGAGAGAGAC	50.5		
ISSR MAO	CTCCTCCTCCTCRC	44		

javanica leaf extract, along with a control. *A. cepa* roots measuring 1.5–3.5 cm were cut and washed multiple times with distilled water. 200 mg of root tissue was weighed and placed in sterile Eppendorf tubes for each treatment and replicate and kept at − 80 °C until DNA isolation. DNA isolation was conducted following a developed CTAB method [32]. To determine the concentration and purity of DNA, all samples were analyzed with a NanoDrop spectrophotometer (Thermo Fisher Scientific, NanoDrop 2000c). Subsequently, each DNA sample was adjusted to a concentration of 25 ng/µL and kept at − 20 °C until PCR amplification.

2.5.2 ISSR-PCR marker

ISSR-PCR was used to detect genotoxicity at the molecular level [33, 34], using an Applied Biosystems VeritiPro PCR machine (USA). Four ISSR primers were utilized for PCR amplification of DNA isolated from *A. cepa* roots. The annealing temperature for each primer was optimized as described in Table 1. The selection of the four ISSR primers (ISSR 418, ISSR-HB12, ISSR-UBS811, and ISSR-MAO) was based on previous studies that successfully used these primers in genetic diversity analysis and genotoxicity evaluation on *A. cepa* [35, 36], as shown in Table 1.

Four samples were used for ISSR-PCR, comprising three samples treated with aqueous *A. javanica* leaf extract and one control. PCR was carried out with the Qiagen Taq PCR Master Mix Kit, which contains $MgCl_2$, dNTPs, Taq polymerase, and reaction buffer. The 20 µL PCR reaction consisted of 10 µL of 2 × master mix, 2 µL of 10 µM primer, 4 µL of DNA template, and a 4 µL aliquot of nuclease-free water. Thermal cycling settings are described in Table 2.

Table 2 Thermal cycler for amplification ISSR-primers

NO	Steps	Temperature (°C)	Time	Cycles
1	Initial denaturation	94	5 min	40
2	Denaturation	94	30 s	
3	Annealing	As per primer	45 s	
4	Extension	72	90 s	
5	Final extension	72	7 min	1
7	Hold	4	To ending program	

2.5.3 Gel electrophoresis

PCR products were stained with 10 µL of SYBR Safe, and gel electrophoresis was performed according to an established protocol [37]. The gel was then immediately placed in a detection system (Bio-Rad) under UV illumination. Images were captured to document and analyze the gels for band detection.

2.6 Analysis of statistical data

The data were assessed using several methods. The MI was determined as (number of dividing cells/total number of cells) × 100 [31]. The percentage of total CA was calculated by using the formula: (total number of CA of different types/total number of cells counted) × 100. EC_{50} values were determined using linear regression analysis in Microsoft Excel [26]. For gel electrophoresis, a dendrogram was generated for the images using the unweighted pair group method with arithmetic mean (UPGMA) algorithm, as implemented in Bio-Rad Quantity One software. Polymorphism was determined by the following equation:

(number of polymorphic bands + number of unique bands/total number of loci) × 100. Results are expressed as mean ± standard deviation (mean ± SD). To compare means, one-way analysis of variance (ANOVA) was conducted with Tukey's post hoc test. The statistical analysis was conducted with IBM SPSS Statistics for Windows, version 29.0.1 (IBM Corp., Armonk, N.Y., USA).

3 Results

3.1 Determination of EC_{50}

Exposure to aqueous *A. javanica* leaf extract for 72 h induced a concentration-dependent inhibition of *A. cepa* root length, as shown in Fig. 3. Increased extract concentration led to a corresponding increase in the inhibition rate. The EC_{50}, defined as the concentration required to inhibit root growth by 50% compared to the control, was calculated to be 26.70 mg/mL, as presented in Fig. 3.

3.2 Growth inhibition of *A. cepa* roots

Figure 4 illustrates the effectiveness of the aqueous *A. javanica* leaf extract in inhibiting *A. cepa* root growth, compared to control, at 24, 48, and 72 h. After 72 h of treatment, root lengths were 29.95 mm for EC_{25} (13.35 mg/mL), 20.80 mm for EC_{50} (26.7 mg/mL), and 13.16 mm for EC_{75} (40.05 mg/mL), compared to 42.16 mm in the control group. A statistically significant decrease in root length ($P < 0.05$) was observed with increasing extract concentration and longer treatment duration, demonstrating a dose-dependent and time-dependent inhibitory effect.

3.3 Cytogenetic analysis

3.3.1 Cytotoxic effects

The MI was calculated to determine cytotoxicity. Table 3 and Fig. 5 illustrate the effects of various concentrations (13.35, 26.7, and 40.05 mg/mL) of aqueous *A. javanica* leaf extract on the division phases of *A. cepa* root meristem cells at exposure times of 24, 48, and 72 h. The data demonstrate

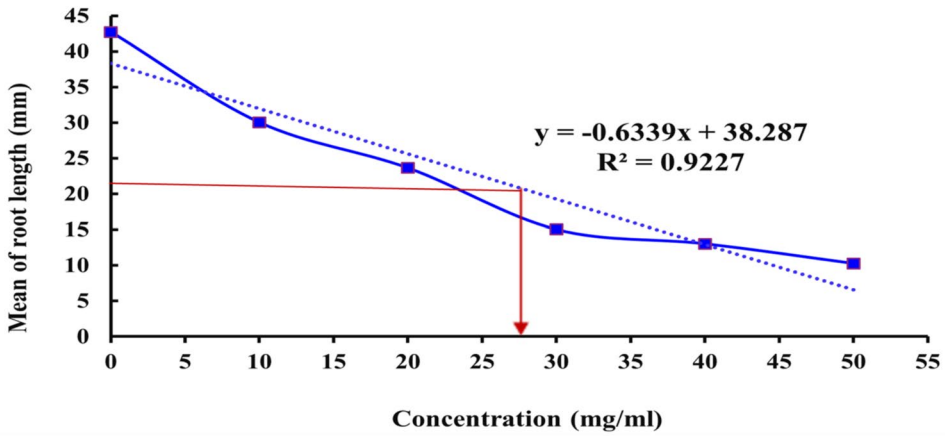


Fig. 3 Linear regression analysis showing variation in root length of A. cepa after treatment with concentrations of aqueous extract of A. javanica leaves; and determination EC50

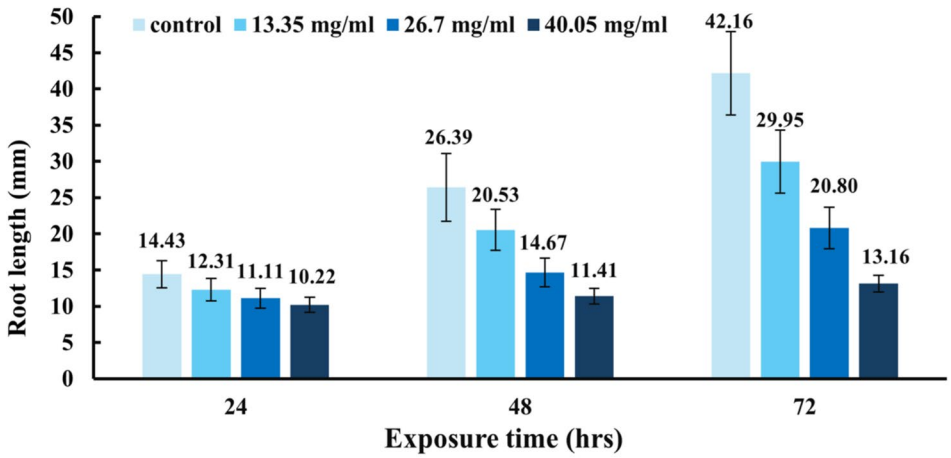


Fig. 4 Effects of aqueous extract of A. javanica leaves on root length of A. cepa after 24, 48, and 72 h

Table 3 Mitotic index (MI) of *A. cepa* root exposed to different period times with various concentrations of aqueous extract of *A. javanica* leaves

ET(hrs.)	Con. (mg/ml)	TCC	TCD	MI %
24	Control	1000	150 ± 3.61	15.00 ± 0.26
	13.35	1000	137 ± 8.54	13.70 ± 0.84
	26.7	1000	129 ± 4.36	12.90 ± 0.38*
	40.05	1000	111 ± 6.08	11.10 ± 0.65*
48	Control	1000	154.33 ± 4.93	15.43 ± 0.52
	13.35	1000	139 ± 5.20	13.90 ± 0.51
	26.7	1000	119.67 ± 8.39	11.97 ± 0.89*
	40.05	1000	82.33 ± 4.04	8.23 ± 0.35*
72	Control	1000	156 ± 2.65	15.60 ± 0.23
	13.35	1000	139.33 ± 3.06	13.93 ± 0.31*
	26.7	1000	105.67 ± 3.21	10.57 ± 0.30*
	40.05	1000	60.33 ± 2.52	6.03 ± 0.22*

ET(hrs.): Exposure time (hrs.), **Con. (mg/ml):** Concentration mg/ml (13.35 = EC_{25}; 26.7 = EC_{50}; 40.05 = EC_{75}), **TCC:** Total cells counted, **TCD:** Total cells division, **MI %:** Mitotic index % ± SD, **(SD):** standard Deviation; **(*):** significant difference at $P < 0.05$ Tukey's HSD test indicating differences compared to control. The values are means ± SD, n = 3

a decrease in the MI of meristem root cells with increasing concentrations of *A. javanica* leaf extract. After 24 h of treatment, the 26.7 and 40.05 mg/mL concentrations exhibited a substantial reduction of the MI to 12.90% and 11.10%, respectively, compared to the 15% observed in the control. Similarly, after 48 h of treatment, the 26.7 and 40.05 mg/mL concentrations indicated a notable decrease in the MI to 11.97% and 8.23%, respectively, compared to 15.43% in the control. Furthermore, after 72 h of treatment, the 13.35, 26.7, and 40.05 mg/mL concentrations each exhibited a remarkable decrease in the MI to 13.93%, 10.57%, and 6.03%, respectively, compared to the control, which was 15.6%.

3.3.2 Genotoxic effects

Table 4 and Fig. 6 present the results of CA and MN. The types of mitotic CA induced by treatment with aqueous *A. javanica* leaf extract included c-metaphase, disturbance, vagrant, fragment, bridge, and stickiness. A significant increase in CA of meristem root cells was observed correlating to the rising concentration of the extract (13.35, 26.7, and 40.05 mg/ml) and prolonged exposure duration ($P < 0.05$ compared to control). The highest percentage of CA was 4.27%, observed in the highest extract concentration of 40.05 mg/ml at 72 h; the predominant abnormalities were sticky chromosomes, followed by MN, vagrant chromosomes, disturbances, c-metaphase, chromosomal bridges, and chromosomal fragments (Fig. 6).

3.4 Molecular analysis

3.4.1 ISSR analysis

ISSR markers were employed to study the extent of genetic variation after exposure to several concentrations of aqueous *A. javanica* leaf extract. Four ISSR primers (ISSR418, ISSR-UBS811, ISSR-HB12, and ISSR-MAO) were used for their specificity and clear bands, as shown in Fig. 7. The number of fragments generated using the four ISSR primers was 43, with an average of 10.75 bands per primer. The polymorphism ratios for ISSR418, ISSR-HB12, ISSR UBS811, and ISSR-MAO, were 100%, 28.57%, 66.66%, 12.5%, respectively (Table 5). In addition, Fig. 8 shows the phylogenetic dendrogram for the four ISSR primers, which shows the variance between the control and concentrations of *A. javanica* extract. The dendrogram emphasizes a dose-dependent response, where the highest concentrations lead to more divergence from the control (Fig. 8).

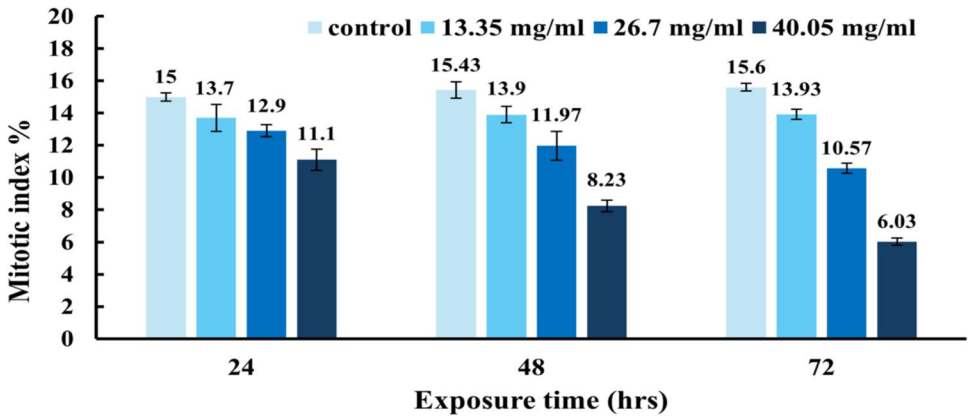


Fig. 5 Mitotic index (MI) of A. cepa meristematic cells over various treatment durations with different concentrations of aqueous extract of A. javanica leaves

Table 4 Percentages of chromosomal aberrations (CA) in meristematic cells of *A. cepa* following treatment with various concentrations of aqueous extract of *A. javanica* leaves for different periods

ET	Con	M %	C-M%	D %	V %	F	B%	S%	CA (%)
24	Control	0.00	0.00	0.07	0.13	0.00	0.00	0.43	0.63 ± 0.25
	13.35	0.00	0.03	0.30	0.40	0.03	0.10	0.63	1.50 ± 0.17*
	26.7	0.03	0.17	0.37	0.57	0.03	0.07	1.27	2.50 ± 0.25*
	40.05	0.30	0.07	0.50	0.67	0.17	0.20	1.23	3.13 ± 0.33*
48	Control	0.00	0.00	0.13	0.27	0.00	0.03	0.53	0.97 ± 0.05
	13.35	0.00	0.07	0.37	0.47	0.03	0.23	0.87	2.03 ± 0.23*
	26.7	0.13	0.10	0.40	0.70	0.07	0.07	1.40	2.87 ± 0.43*
	40.05	0.53	0.13	0.30	0.67	0.23	0.27	1.50	3.63 ± 0.21*
72	Control	0.00	0.03	0.17	0.27	0.03	0.03	0.43	0.97 ± 0.15
	13.35	0.10	0.20	0.23	0.70	0.03	0.07	1.17	2.50 ± 0.18*
	26.7	0.23	0.20	0.43	0.97	0.07	0.17	1.57	3.63 ± 0.07*
	40.05	1.00	0.20	0.47	0.77	0.13	0.17	1.53	4.27 ± 0.18*

ET(hrs.): Exposure time (hrs.), **Con.:** Concentration (mg/ml), **M%:** Micronucleus%, **C-M%:** C-Metaphase %, **D %:** Disturbance %, **V %:** Vagrant%, **F:** Fragments, **B %:** Bridge %, **S%:** Stickiness%, **CA%:** Chromosomal aberration % ± SD, **(SD):** standard Deviation; **(*):** significant difference at $P < 0.05$ by Tukey's HSD test indicating differences compared to control. The values are means ± SD, n = 3

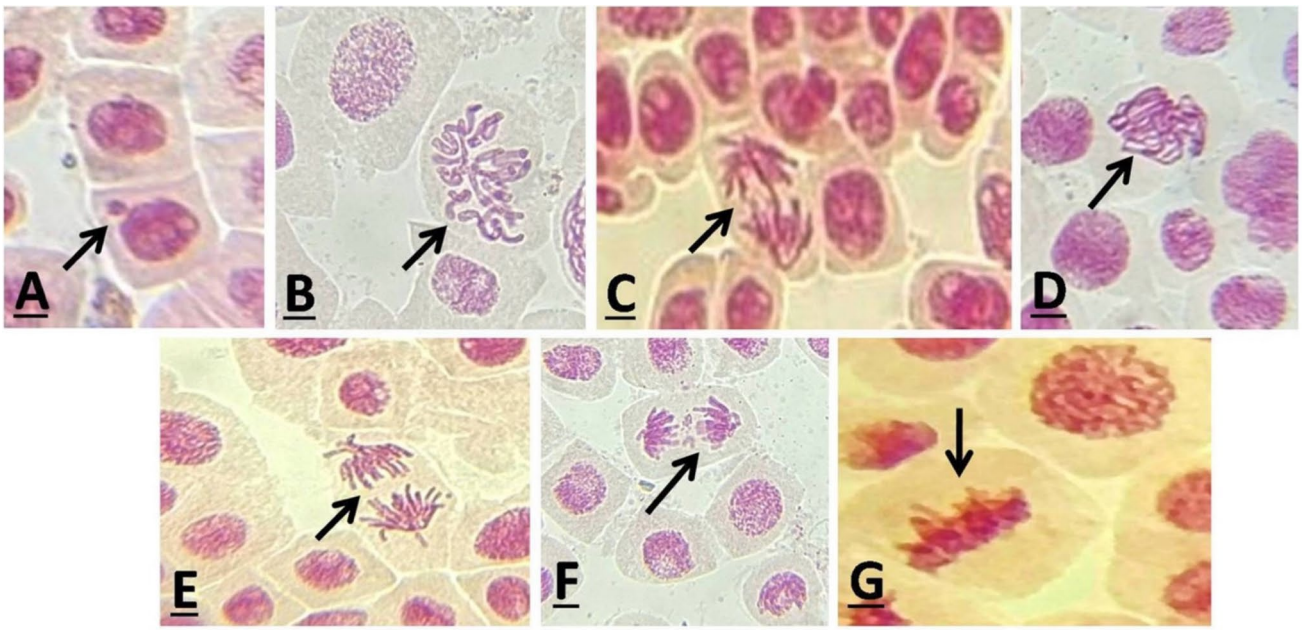


Fig. 6 Different types of chromosomal aberration (CA) after treated **A** Micronucleus in interphase, **B** C-Metaphase, **C** Anaphase bridge, **D** Disturbance, **E** Chromosome vagrant in anaphase, **F** Chromosome fragments in anaphase, **G** Stickiness in metaphase

4 Discussion

This study evaluated the potential genotoxicity of an aqueous *A. javanica* leaf extract on *A. cepa* roots using cytogenetic and molecular assays. EC_{50} has previously proven to be a useful standard for determining test concentrations when evaluating genotoxicity [38, 39]. The aqueous *A. javanica* leaf extract demonstrated a 50% decrease in *A. cepa* root growth compared to the control at a concentration of 26.7 mg/mL. We consequently included test concentrations below (13.35 mg/mL) and above (40.05 mg/mL) this EC_{50}. The *A. cepa* root growth inhibition test demonstrated a significant dose-dependent inhibition, indicating possible cytotoxicity of the extract. The suppression of root growth in the *A. cepa* assay may be attributable to apical meristematic activity [40, 41], inhibition of cell elongation in elongation zones [42, 43], and inhibition of protein synthesis [38].

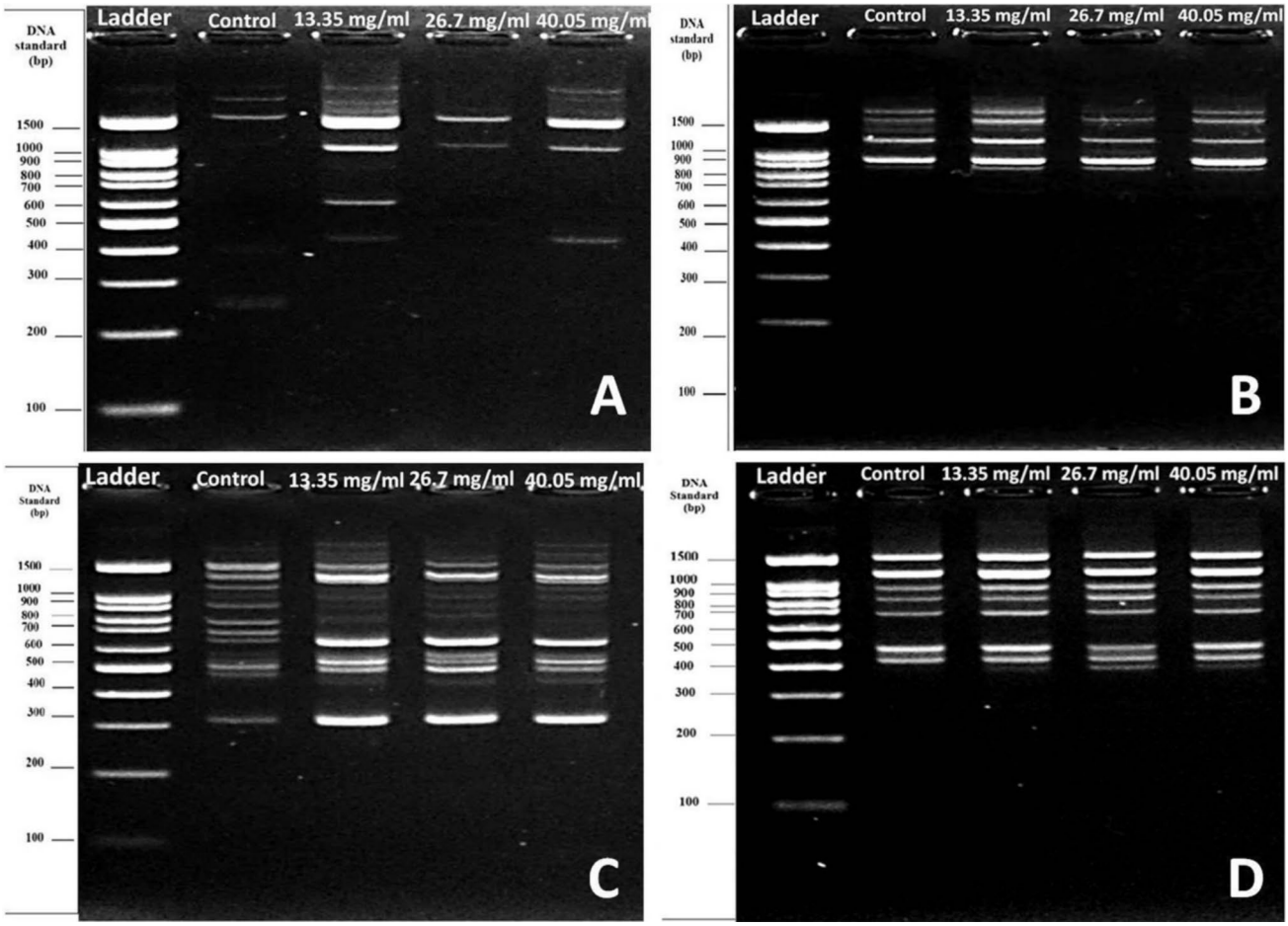


Fig. 7 Gel electrophoresis bands of ISSR-PCR products of DNA from A. cepa root treated with various concentrations of aqueous extract of A. javanica and control. **A** ISSR418, **B** ISSR-HB12, **C** ISSR-UBS 811, and **D** ISSR-MAO

Table 5 Primer data analysis of ISSR of DNA treated with various concentrations of aqueous extract of *A. javanica* and control

No	PN	Primer's sequence 5′–3′	Tm	TB	SR (bp)	TPB	P %
1	418	CTCTCTCTCTCTCTCTTG	51	10	253–2394	10	100%
2	HB12	CAGCAGCAGGC	36	7	886–1883	2	28.57%
3	UBC-811	GAGAGAGAGAGAGAGAC	50.5	18	315–2018	12	66.66%
4	MAO	CTCCTCCTCCTCRC	44	8	396–1575	1	12.5%
Total					25	51.93	

PN: Primer name; **Tm:** Annealing temperature; **TB:** Total bands; **SR:** Size range (bp); **TPB:** Total polymorphic bands; **P%:** Polymorphism %

We also demonstrated that MI values at 72 h significantly decreased with exposure to EC_{25}, EC_{50}, and EC_{75} concentrations of the *A. javanica* leaf extract in *A. cepa* meristematic cells, reaching 13.93%, 10.57%, and 6.03%, respectively, compared to a control value of 15.60%. Higher concentrations of the *A. javanica* extract led to greater reductions in MI. Consequently, the reduction in the length of roots can be attributed to a decreased rate of mitotic division, indicating cytotoxicity [44]. The MI at the highest concentration, EC_{75} (40.05 mg/mL), decreased below 50% of the control. A prior investigation [45] indicated that a reduction in MI to 20% results in sublethal effects on the tested organisms, while a decrease to 50% leads to lethal effects [46]. The significant decrease in MI may result from inhibition of DNA synthesis, suppression of DNA synthesis during the G1 phase, or interference with the G2 phase of the cell cycle by the test substance [47–49].

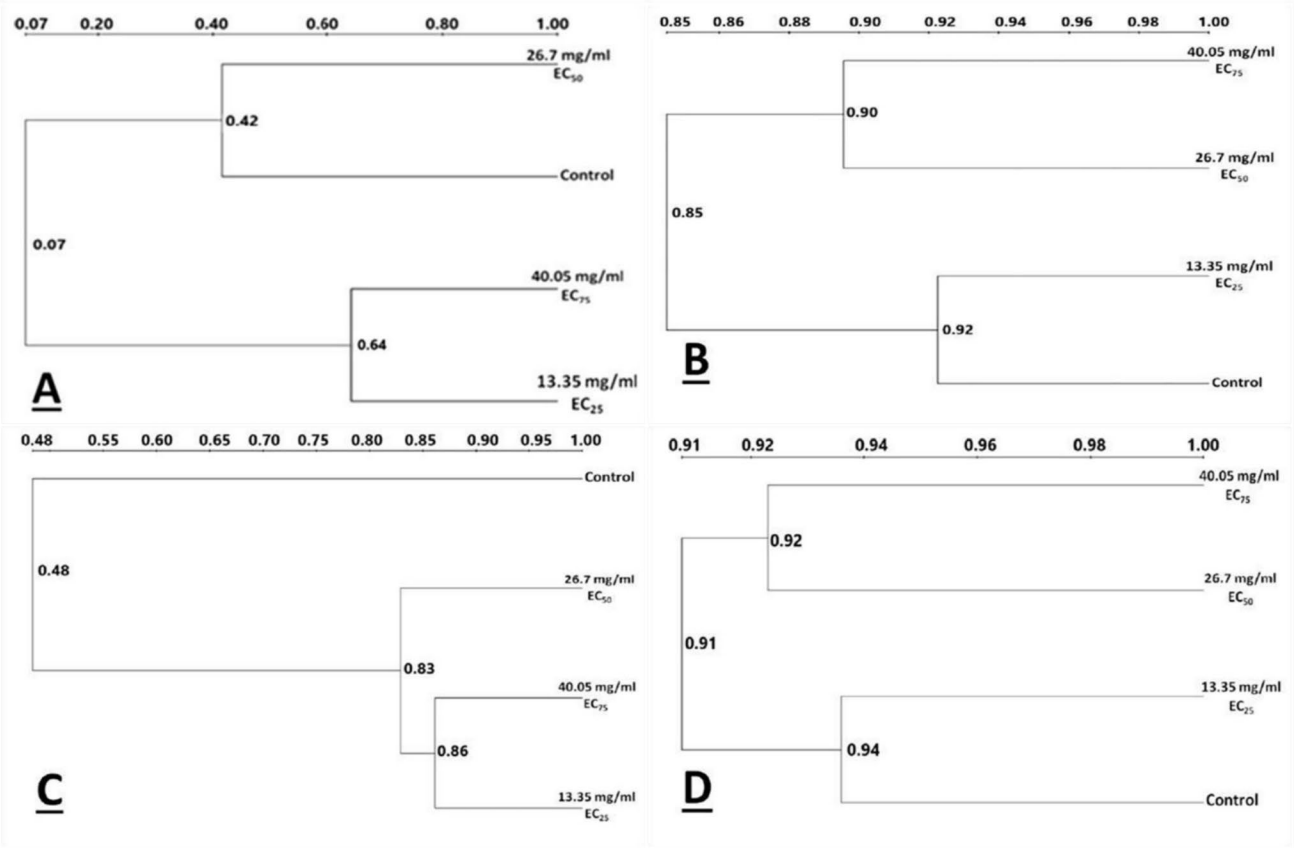


Fig. 8 The phylogenetic tree based on the ISSR marker for various concentrations of aqueous extract of A. javanica compared to the control. **A** ISSR418, **B** ISSR-HB12, **C** ISSR-UBS 811, and **D** ISSR-MAO

Treatment with the aqueous *A. javanica* leaf extract at various concentrations and exposure periods also remarkably increased the occurrence of CA compared to the control. The MN frequency also increased with increasing concentration. MN are chromosomal fragments resulting from mitotic segregation errors [50] or unrepaired DNA breaks [51]. Various abnormalities were observed, including sticky chromosomes, MN, vagrant chromosomes, disturbances, c-metaphase, chromosomal fragments, and chromosomal bridges. Sticky chromosomes were the most frequent CA. Stickiness results from either enhanced condensation or contraction of chromosomes, or partial dissolution of nucleoproteins and DNA depolymerization. This form of CA indicates deleterious consequences that are often permanent and may lead to cell death [52]. Pathiratne et al. found vagrant chromosomes to be readily identifiable in *A. cepa* root meristematic cells [53], related to an unbalanced distribution of chromosomes [54]. Another observed abnormality was c-mitosis, which suggests the reduction of spindle formation in metaphase. The occurrence of c-mitosis indicates the presence of a microtubule toxin [55]. These aberrations may also result from spindle failure caused by the interaction of phytochemical components with the spindle apparatus [56]. Similar to our results, Chowdhury & Biswas reported that leaf extract of *Mucuna bracteata* exhibited genotoxicity and cytotoxicity in *A. cepa*, evidenced by a decreased MI and various CA, including disordered prophase, sticky chromosomes, chromosomal bridges, and lagging chromosomes. These effects were also dose-dependent [57].

ISSR markers indicated DNA damage in response to several concentrations of the aqueous *A. javanica* leaf extract. The outcomes of the molecular and cytogenetic analyses were generally consistent. The polymorphism ratios for the extract were 100%, 28.57%, 66.66%, and 12.5% for primers ISSR418, ISSR-HB12, ISSR-UBS811, and ISSR-MAO, respectively. Alterations were observed as the gain and/or loss of bands among the various concentrations compared to control. This shift in ISSR banding patterns, especially the gain or loss of bands, is linked to DNA mutations or damage at primer binding sites [58]. These molecular changes are consistent with our cytogenetic results, including reduced MI and increased CA, supporting the evidence of genotoxic effects of the extract at both the chromosomal and DNA levels.

A dendrogram produced utilizing the UPGMA algorithm based on the ISSR-PCR analysis demonstrated genetic

variation between the concentrations of the *A. javanica* leaf extract and the control. UPGMA analysis is used to assess genetic variation within and between groups [59]. These results suggest that an aqueous *A. javanica* leaf extract may induce genetic mutations. It has been previously demonstrated that certain plant-based remedies include harmful substances. Frequently, these substances interact with biological molecules within cells, such as DNA, and can cause cellular damage or genetic mutations [60]. Mutations can influence primer annealing, while DNA damage might disrupt the activity of DNA polymerase, consequently affecting the quantity of newly synthesized amplicons [61]. In a similar study, the genotoxic effect of a methanolic leaf extract of *Forsskaolea tenacissima* L. on *A. cepa* was evaluated using ISSR-PCR molecular markers; high concentrations of the *F. tenacissima* leaf methanol extract were shown to cause genetic mutations [35].

While this study presents strong and consistent evidence on the genotoxic and cytotoxic effects of *A. javanica* aqueous leaf extract, using both cytogenetic and molecular assays, it is important to state that our results are derived from a plant-based model. The *A. cepa* assay and ISSR-PCR markers used in this work are well-established and widely accepted tools in genotoxicity research; however, applying additional biological models and more comprehensive molecular techniques in future studies could offer a broader understanding.

5 Conclusions

This study is among the first to analyze the genotoxic and cytotoxic effects of *A. javanica* aqueous leaf extract using cytogenetic and molecular (ISSR-PCR) assays on *A. cepa* meristematic cells. The extract caused a dose-dependent reduction in root growth and mitotic activity, and significantly increased chromosomal abnormalities and MN. ISSR analysis revealed high polymorphic banding patterns, confirming DNA-level alterations. These findings suggest that the extract may have genotoxic potential, and careful evaluation is needed before considering its use in therapeutic applications. Further studies should include animal or human cells to confirm whether similar effects occur in non-plant contexts. Additionally, phytochemical screening of samples collected from the Makkah region is recommended to better associate the biological effects with specific bioactive compounds.

Acknowledgements The authors gratefully acknowledge Umm Al-Qura University for providing access to its laboratory facilities.

Author contributions AFA Conceptualization and Supervision; MMA Data curation and Investigation, MMA, HOS Data analysis and Draft preparation; AFA, MMA, HOS Revision and Editing.

Funding This project does not receive any fund.

Data availability All data supporting the findings of this study are available within the paper.

Declarations

Conflict of interest All authors declare that there are no conflicts of interest related to the research presented in this manuscript.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

1. Chaachouay N, Zidane L (2024) Plant-derived natural products: a source for drug discovery and development. Drugs Drug Candidates 3(1):184–207. https://doi.org/10.3390/ddc3010011
2. Freire CDJ, Barbosa LRS, Costa JG, Santos RGA, Santos AFD (2018) Phytotherapy in pediatrics: the production of knowledge and practices in primary care. Rev Bras Enferm 71(suppl 1):637–645. https://doi.org/10.1590/0034-7167-2017-0436
3. Tounekti T, Mahdhi M, Khemira H (2019) Ethnobotanical study of indigenous medicinal plants of Jazan region. Saudi Arabia Ecam 201(3):1–45. https://doi.org/10.1155/2019/3190670
4. Al Akeel M, Al Ghamdi W, Al Habib S, Koshm M, Al Otaibi F (2018) Herbal medicines: Saudi population knowledge, attitude, and practice at a glance. J Family Med Prim Care 7(5):865. https://doi.org/10.4103/jfmpc.jfmpc_315_17
5. Shahin SM, Jaleel A, Alyafei MAM (2021) Yield and in vitro antioxidant potential of essential oil from *Aerva javanica* (Burm f.) juss. ex schul. flower with special emphasis on seasonal changes. Plants 10:12. https://doi.org/10.3390/plants10122618
6. Madhusudhan K, Subramanyam S, Jayaveera K (2023) A Review on phytochemistry and biological activities of *Aerva javanica* (Burm f.) Juss. ex Schult. Phytochem Comp Pharma Med Plants 2:145
7. Arbab AH, Parvez MK, Al-Dosari MS, Al-Rehaily AJ, Ibrahim KE, Alam P, Alsaid MS, Rafatullah S (2016) Therapeutic efficacy of ethanolic extract of *Aerva javanica* aerial parts in the amelioration of CCl4-induced hepatotoxicity and oxidative damage in rats. Food Nutr Res 60:30864. https://doi.org/10.3402/FNR.V60.30864
8. Khan AW, Jan S, Parveen S, Khan RA, Saeed A, Tanveer AJ, Shad AA (2012) Phytochemical analysis and enzyme inhibition assay of *Aerva javanica* for ulcer. Chem Cent J 6(1):76. https://doi.org/10.1186/1752-153x-6-76
9. Karela P, Dedar RK (2022) Phytochemistry and pharmacological aspect of *Areva javanica*: A review. J Pharm Innov 11(7):1373–1375
10. Anand SP, Velmurugan G, Keerthiga M, Nandagopalan V (2018) A comparative study on antimicrobial activity of Argemone mexicana Linn. and Aerva javanica (Burm. F.) Juss. Ex. Schult. Am J PharmTech Res 4(1):531–539

11. Movaliya V, Zaveri M (2014) Evaluation of nephro-protective effect of different fractions of alcoholic extract of root of *Aerva Javanica*. Int J Pharm Sci Rev Res 25(2):280–286
12. Saeed H, Nawaz H, Shad MA, Shahwar DE, Andaleeb H, Muzaffar S, Jabeen R, Rehman T, Waheed A (2019) Antioxidant potential of cell wall polysaccharides extracted from various parts of *Aerva javanica*. Free Radic Antioxid 9(1):35–42. https://doi.org/10.5530/fra.2019.1.7
13. Swarnkar SK, Khunteta A, Paliwal S (2016) In vitro antioxidant potential of aqueous extract of *Calligonum polygonoides* seeds by ABTS and DPPH Assay. In: 11th International conference-BICON, Jaipur, Rajasthan, India.
14. Joanofarc J, Vamsadhara C (2003) Evaluation of antidiarrhoeal activity of *Aerva* species. Nat Prod Sci 9(3):177–179
15. Al-Shehri M, Moustafa M (2019) Anticancer, antibacterial, and phytochemicals derived from extract of *Aerva javanica* (Burm.f.) Juss. ex Schult. grown naturally in Saudi Arabia. Trop Conserv Sci 12:2019. https://doi.org/10.1177/1940082919864262
16. Movaliya V, Zaveri M (2012) Pharmacognostical studies on roots of *Aerva javanica*. Int J Pharm Sci Rev Res 16(1):34–37
17. Thotathil V, Sidiq N, Fakhroo A, Sreerama L (2023) Phytochemical analysis of *Anastatica hierochuntica* and *Aerva javanica* grown in Qatar: their biological activities and identification of some active ingredients. Molecules 28(8):2023. https://doi.org/10.3390/molecules28083364/s1
18. Anand SP, Doss A, Nandagopalan V (2014) Qualitative and quantitative analysis of phytochemicals in *Aerva javanica* (Burm. F.) Schult. Acta Biomed Sci 1(2):93–97
19. Kahaliw W, Hellman B, Engidawork E (2018) Genotoxicity study of Ethiopian medicinal plant extracts on HepG2 cells. BMC Complement Altern Med 18(1):1–8. https://doi.org/10.1186/s12906-017-2056-x/figures/3
20. Romero-Jiménez M, Campos-Sánchez J, Analla M, Muñoz-Serrano A, Alonso-Moraga A (2005) Genotoxicity and anti-genotoxicity of some traditional medicinal herbs. Mutat Res Genet Toxicol Environ Mutagen 585(1–2):147–155. https://doi.org/10.1016/j.mrgentox.2005.05.004
21. Maroyi A (2023) Medicinal uses of the Fabaceae Family in Zimbabwe: a review. Plants 12(6):2023. https://doi.org/10.3390/plants12061255
22. de Melo EC, Pinheiro RS, Costa BS, Lima RMT, Dias ACS, Santos TJA, Nascimento MLLB, Sousa JMC, Islam MT, Cavalcante AACM, El-Nashar HAS, El-Shazly M, Filho JWGO (2024) *Allium cepa* as a toxicogenetic investigational tool for plant extracts: a systematic review. Chem Biodivers 21(12):e202401406. https://doi.org/10.1002/cbdv.202401406
23. Correia S, Matos M, Ferreira V, Martins N, Gonçalves S, Romano A, Pinto-Carnide O (2014) Molecular instability induced by aluminum stress in Plantago species. Mutat Res Genet Toxicol Environ Mutagen 770:105–111. https://doi.org/10.1016/j.mrgentox.2014.06.002
24. Marbaniang C, Malemnganbi Chanu T, Chanu TM (2023) Determination of the safe dose of aqueous extract of *Apium graveolens* L. By acute and sub-acute toxicity study. J Plant Sci Res 10(2):252
25. Çelik TA, Aslantürk ÖS (2010) Evaluation of cytotoxicity and genotoxicity of *Inula viscosa* leaf extracts with *Allium* test. J Biomed Biotechnol 2010:189252. https://doi.org/10.1155/2010/189252
26. Qari S (2016) Cytotoxic and genotoxic assessment of *Citrullus colocynthis*. Int J Sci Res 5(2):20–39
27. Kalcheva VP, Dragoeva AP, Kalchev KN, Enchev DD (2009) Cytotoxic and genotoxic effects of Br-containing oxaphosphole on *Allium cepa* L. root tip cells and mouse bone marrow cells. Genet Mol Biol 32(2):389–393
28. Yekeen A, Adeboye T, Messach K (2013) Cytogenotoxic effects of cypermethrin, deltamethrin, lambda cyhalothrin and endosulfan pesticides on *Allium cepa* root cells. Afr J Biotechnol 12(41):6000–6006. https://doi.org/10.5897/ajb2013.12802
29. Renjith Raju A, George Paul U, Aguilor P, Capili JT (2021) The effect of induced acid rain; *Allium cepa* chromosomal aberration assay. Scholars Acad J Biosci 9(3):89–97. https://doi.org/10.36347/sajb.2021.v09i03.005
30. Grant WF (1982) Chromosome aberration assays in *Allium* A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat Res 99(3):273–291
31. Fiskesjö G (1985) The *Allium* test as a standard in environmental monitoring. Hereditas 102(1):99–112. https://doi.org/10.1111/j.1601-223.1985.tb00471.x
32. Aboul-Maaty NAF, Oraby HAS (2019) Extraction of high-quality genomic DNA from different plant orders applying a modified CTAB-based method. Bull Nat Res Cen 43(1):1–10. https://doi.org/10.1186/s42269-019-0066-1
33. Poyraz I (2022) An investigation of the genotoxic and cytotoxic effects of myclobutanil fungicide on plants. Plant Protec Sci 58(1):57–64. https://doi.org/10.17221/6/2021-pps
34. Poyraz İE, Poyraz İ, Kıyan HT, Öztürk N, Erken S, Gülbağ F, Özzambak ME (2018) Detection of the genotoxicity of *Gentiana* L. extracts by using RAPD-PCR and ISSR-PCR techniques. Indian J Pharma Educ Res 52(4):S133–S139. https://doi.org/10.5530/ijper.52.4s.89
35. Qari SH, Ashour AB, Attia KA, Alrefaei AF, Filfilan WM, Elbeeh ME, Qumsani AT (2023) Genotoxicity and carcinogenicity of *Frosskaolea tenacissima (Linn)* leave extract used in traditional medicine in Saudi Arabia. J Innov Sci Inform Ser Net 20(3):789–799
36. Sudha GS, Ramesh P, Akila CS, Talla SK, Chari B, Riazunnisa K (2018) Genetic diversity analysis of selected Onion (*Allium cepa* L.) germplasm using specific RAPD and ISSR polymorphism markers. Biocatal Agric Biotechnol 17:605–613. https://doi.org/10.1016/j.bcab.2018.11.007
37. Balletbó A, Bol S (2019) Agarose gel electrophoresis V2. https://doi.org/10.17504/protocols.io.7t5hnq6.
38. Chauhan LKS, Saxena PN, Gupta SK (1999) Cytogenetic effects of cypermethrin and fenvalerate on the root meristem cells of *Allium cepa*. Environ Exp Bot 42(3):181–189. https://doi.org/10.1016/s0098-8472(99)00033-7
39. Seth CS, Chaturvedi PK, Misra V (2007) Toxic effect of arsenate and cadmium alone and in combination on giant duckweed (*Spirodela polyrrhiza* L.) in response to its accumulation. Environ Toxicol 22(6):539–549. https://doi.org/10.1002/tox.20292
40. Liu D, Jiang W, Gao X (2003) Effects of cadmium on root growth, cell division and nucleoli in root tip cells of garlic. Biol Plant 47(1):79–83. https://doi.org/10.1023/a:1027384932338/metrics
41. Webster P, MacLeod R (1996) The root apical meristem and its margin. In: Waishel Y, Eshel A, Kafkafi U (eds) The hidden half, 2nd edn. Marcel Dekker, New York, pp 51–76
42. Fusconi A, Repetto O, Bona E, Massa N, Gallo C, Dumas-Gaudot E, Berta G (2006) Effects of cadmium on meristem activity and nucleus ploidy in roots of *Pisum sativum* L. cv. frisson seedlings. Environ Exp Bot 58(1–3):253–260. https://doi.org/10.1016/j.envexpbot.2005.09.008
43. Voutsinas G, Zarani FE, Kappas A (1997) The effect of environmental aneuploidy-inducing agents on the microtubule architecture of mitotic meristematic root cells inhordeum vulgare. Cell Biol Int 21(7):411–418. https://doi.org/10.1006/cbir.1997.0171
44. Owolarafe TA, Salawu K, Ihegboro GO, Ononamadu CJ, Alhassan AJ, Wudil AM (2020) Investigation of cytotoxicity potential of different extracts of *Ziziphus mauritiana* (Lam) leaf *Allium cepa* model. Toxicol Rep 7:816. https://doi.org/10.1016/j.toxrep.2020.06.010

45. Antosiewicz D (1990) Analysis of the cell cycle in the root meristem of *Allium cepa* under the influence of ledakrin. Folia Histochem Cytobiol 28(1–2):79–95
46. Panda PP, Sahu UK (1885) Induction of abnormal spindle function and cytokinesis inhibition in mitotic cells of *Allium cepa* by the organophosphorus insecticide fensulfothion. Cytobios 42:147–155
47. Majewska A, Wolska E, Kuraś M, Śliwińska E, Furmanowa M, Urbańska N, Pietrosiuk A, Zobel A (2003) Antimitotic effect, G2/M accumulation, chromosomal and ultrastructure changes in meristematic cells of *Allium cepa* L. root tips treated with the extract from *Rhodiola rosea* roots. Caryologia 56(3):337–351. https://doi.org/10.1080/00087114.2003.10589343
48. El-Ghamery AA, El-Nahas AI, Mansour MM (2000) The action of atrazine herbicide as an inhibitor of cell division on chromosomes and nucleic acids content in root meristems of *Allium cepa* and *Vicia faba*. Cytologia 65(3):277–287. https://doi.org/10.1508/cytologia.65.277
49. Sudhakar R, Ninge Gowda KN, Venu G (2001) Mitotic abnormalities induced by silk dyeing industry effluents in the cells of *Allium cepa*. Cytologia 66(3):235–239. https://doi.org/10.1508/cytologia.66.235
50. Fenech M, Kirsch-Volders M, Natarajan AT, Surralles J, Crott JW, Parry J, Norppa H, Eastmond DA, Tucker JD, Thomas P (2011) Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. Mutagenesis 26(1):125–132. https://doi.org/10.1093/mutage/geq052
51. Ly P, Teitz LS, Kim DH, Shoshani O, Skaletsky H, Fachinetti D, Page DC, Cleveland DW (2016) Selective Y centromere inactivation triggers chromosome shattering in micronuclei and repair by canonical NHEJ. Nat Cell Biol 19(1):68. https://doi.org/10.1038/ncb3450
52. Türkoğlu S (2007) Genotoxicity of five food preservatives tested on root tips of *Allium cepa* L. Mutat Res Genet Toxic Environ Mutagen 626(1–2):4–14. https://doi.org/10.1016/j.mrgentox.2006.07.006
53. Pathiratne A, Hemachandra CK, De Silva N (2015) Efficacy of *Allium cepa* test system for screening cytotoxicity and genotoxicity of industrial effluents originated from different industrial activities. Environ Monit Assess 187(12):1–12. https://doi.org/10.1007/s10661-015-4954-z
54. Sondhi N, Bhardwaj R, Kaur S, Kumar N, Singh B (2008) Isolation of 24-epibrassinolide from leaves of *Aegle marmelos* and evaluation of its antigenotoxicity employing *Allium cepa* chromosomal aberration assay. Plant Growth Regul 54(3):217–224. https://doi.org/10.1007/s10725-007-9242-7
55. Shahin SA, El-Amoodi KH (1991) Induction of numerical chromosomal aberrations during DNA synthesis using the fungicides nimrod and rubigan-4 in root tips of *Vicia faba* L. Mutat Res Gen Toxic 261(3):169–176. https://doi.org/10.1016/0165-1218(91)90064-s
56. Ihegboro GO, Alhassan AJ, Ononamadu CJ, Owolarafe TA, Sule MS (2020) Evaluation of the biosafety potentials of methanol extracts/fractions of Tapinanthusbangwensis and *Moringa oleifera* leaves using *Allium cepa* model. Toxicol Rep 7:671–679. https://doi.org/10.1016/j.toxrep.2020.05.001
57. Chowdhury MA, Biswas A (2024) Evaluation of cyto-genotoxic effects of Mucuna Bracteata DC. Ex Kurz leaf extracts by *Allium Cepa*. J Biosci 32(2):71–82. https://doi.org/10.3329/jbs.v32i2.75760
58. Aly AA, Eliwa NE, Maraei RW (2019) Genetic variation based on ISSR in two wheat cultivars after exposing to gamma radiation. ScienceAsia 45(5):436–445. https://doi.org/10.2306/scienceasia1513-1874.2019.45.436
59. Wang J, Dai W, Chen J, Ye K, Lai Q, Zhao D (2023) Assessment of Genetic diversity and genetic structure of *Saussurea medusa* (Asteraceae), a 'Sky Island' Plant in the Qinghai-Tibet Plateau, using SRAP Markers. Plants 12(13):2463. https://doi.org/10.3390/plants12132463/s1
60. Rietjens IMCM, Martena MJ, Boersma MG, Spiegelenberg W, Alink GM (2005) Molecular mechanisms of toxicity of important food-borne phytotoxins. Mol Nutr Food Res 49(2):131–158. https://doi.org/10.1002/mnfr.200400078
61. Atienzar FA, Jha AN (2004) The random amplified polymorphic DNA (RAPD) assay to determine DNA alterations, repair and transgenerational effects in B(a)P exposed Daphnia magna. Mutat Res Fund Mol Mech Mutagen 552(1–2):125–140. https://doi.org/10.1016/j.mrfmmm.2004.06.009

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.