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Article

Genetic Toxicity Effects of Aqueous and Alcoholic Extracts of *Alhagi maurorum* on Onion (*Allium cepa*) Root Tip Cells Using PCR Analysis

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Abstract: Medicinal plants play an important role in traditional and modern medicine due to their wide range of pharmacological properties. Despite their therapeutic value, some plant-derived compounds may exhibit cytotoxic or genotoxic effects depending on their chemical composition and concentration (Wink, 2010; Cowan, 1999; Ali et al., 2021). Therefore, evaluating the safety of medicinal plants has become an important component of toxicological research.

The *Allium cepa* assay is widely used as a reliable biological system for detecting cytotoxicity and genotoxicity. Onion root meristematic cells divide rapidly and respond sensitively to chemical exposure (Fiskesjö, 1985; Leme & Marin-Morales, 2009; Silva et al., 2020)., making them suitable indicators of chromosomal damage and DNA alterations.

The emergence and disappearance of bundles in the genome of onion plant *Allium cepa* .

In this study, the genetic toxicity of aqueous and alcoholic extracts of *Alhagi maurorum* was investigated using onion (*Allium cepa*) root tip cells. Aqueous extract concentrations included 15%, 20%, 25%, 35%, and 45%, while alcoholic extract concentrations included 2%, 3%, 4%, 6%, and 8%. Polymerase Chain Reaction (PCR) analysis was applied to detect possible DNA alterations associated with exposure to these plant extracts (Williams et al., 1990; Atienzar & Jha, 2006; Iqbal et al., 2020).

Keywords: *Alahgi mauorum.* Medicinal plants. Genetic polymorphism.

Introduction

The traditional medicine systems of various cultures have used medicinal plants for thousands of years. Many modern pharmaceutical drugs originate from plant-derived secondary metabolites such as flavonoids, alkaloids, phenolic compounds, and terpenoids. These bioactive compounds exhibit a wide range of biological activities, including antimicrobial, antioxidant, and anti-inflammatory effects [1][2][3].

Medicinal plants provide positive effects, but their excessive consumption results in negative health effects. The term genotoxicity describes the capacity of chemical substances to induce damage to genetic material [3][4], which results in DNA mutations and chromosomal abnormalities and leads to genomic instability [5][6][7].

The *Allium cepa* test system has become an internationally recognized experimental model for genotoxicity assessment because it offers three essential qualities of testing effectiveness through its simple design and dependable performance and its ability to detect minute genetic changes. Researchers can easily identify chromosomal abnormalities because onion root tip meristematic cells undergo rapid cell division while their large chromosomes enable detection of chromosomal defects [8]

The current research demonstrates that scientists need to combine molecular techniques, which include RAPD-PCR, with their cytogenetic research methods to identify DNA damage at the molecular level [9][10].

The plant *Alhagi maurorum* which people refer to as camel thorn belongs to the Fabaceae family as a perennial species. The plant grows throughout the arid and semi-arid regions of the Middle East and North Africa because people have used it since ancient times to treat kidney stones and digestive disorders and inflammatory diseases [11][12]

The previous research showed that multiple plant extracts can affect both the mitotic process and the chromosome structure of onion root cells. Fiskesjö [13] established the Allium test as a standard biological method for environmental monitoring.

Akinboro and Bakare discovered that various medicinal plant extracts caused chromosomal abnormalities in onion root cells through the induction of chromosome stickiness bridges and lagging chromosomes and micronuclei formation [14].

Grant demonstrated that higher plant bioassays function as vital tools for detecting environmental mutagens while assessing the cytogenetic impact of chemical substances [15]. Recent research studies have utilized molecular techniques which include PCR analysis to identify DNA changes that toxic compounds induce. The molecular methods provide additional information about genotoxic mechanisms while they support cytological research.

In the study , genotoxicity was detected using the DNA of the onion *Aliium cepa* . root as a biomarker to confirm the genotoxicity of *Alhagi maurorum.*

Materials and Method

The researchers selected healthy onion bulbs which belong to the *Allium cepa* species and they let the bulbs sprout in distilled water until the roots developed to a length of 2 to 3 centimeters. The researchers used standard extraction methods to produce both aqueous and alcoholic extracts from *Alhagi maurorum*.

The experiment used five aqueous extract concentrations which were 15% 20% 25% 35% and 45% and the experiment used five alcoholic extract concentrations which were 2% 3% 4% 6% and 8%. The control samples were kept in distilled water.

DNA extraction from root tips occurred after researchers collected the samples following their exposure to the tested substances [16][17][18]. The researchers used specific primers to perform PCR amplification [19][20] which allowed them to study the resulting DNA fragments through agarose gel electrophoresis to identify any genetic damage that may have occurred. The spectrophotometric analysis through A260/A280 ratio assessment confirmed DNA quality which needed to pass the test for PCR amplification according to [21].

Result and Discussion

Results

Plant DNA Extraction Method

Researchers performed DNA extraction from *Allium cepa* root tip tissues by using the Cetyltrimethylammonium Bromide method. The method serves as a standard procedure for plant

DNA extraction because it successfully eliminates polysaccharides and secondary metabolites which could disrupt molecular analysis.

The researchers collected 100 mg of fresh Allium cepa root tip tissue and ground it into a fine powder using liquid nitrogen with a sterile mortar and pestle. The powdered tissue was then transferred into a microcentrifuge tube containing CTAB extraction buffer composed of 2% CTAB, 100 mM Tris-HCl (pH 8.0), 20 mM EDTA, 1.4 M NaCl, 1% polyvinylpyrrolidone (PVP), and 0.2% β-mercaptoethanol, which is widely used for plant DNA isolation due to its efficiency in removing polysaccharides and phenolic compounds (CTAB DNA extraction method) [22][23].

The mixture was incubated at 65°C for 30–60 minutes to lyse the cells and release nucleic acids. The incubation was completed after which chloroform:isoamyl alcohol (24:1) was added in equal volume to remove proteins and other contaminants. The mixture was spun in a centrifuge at 12000 rpm for 10 minutes.

The aqueous phase was transferred to a new tube and DNA was precipitated by cold isopropanol followed by centrifugation. . The DNA pellet was washed with 70% ethanol, air-dried, and dissolved in TE buffer. The extracted genomic DNA was stored at −20°C until further use in PCR analysis.

DNA Concentration and Quantification

The concentration and purity of extracted genomic DNA were measured using a UV spectrophotometer at wavelengths of 260 nm and 280 nm. DNA concentration was calculated from the absorbance value at 260 nm, where an absorbance value of 1.0 corresponds to approximately 50 μg/mL of double-stranded DNA. DNA purity was evaluated using the A260/A280 ratio. A ratio between 1.8 and 2.0 indicates high-quality DNA suitable for PCR amplification and molecular analysis. DNA samples with lower ratios may indicate protein contamination, while higher ratios may indicate RNA contamination. Typical DNA concentrations obtained from plant tissues using the CTAB method range between 5–30 μg per sample depending on the plant species and tissue type. The extracted DNA was diluted to suitable working concentrations before being used for PCR analysis to evaluate possible genetic toxicity effect [24][25].

PCR-RAPD Technique

Primers use in RAPD-PCR reaction shown in table (1) arbitrary primers supplied by intergrati DNA Technology . and poiymerase chain reaction PCR program shown in table (2)

Table 1. primers used in this study

peimer sequence	Primer	NO
5'-CAGGCCCTTC-3'	OPA-01	1
5'-TGCCGAGCTG-3'	OPA-02	2
5'-AGTCAGCCAC-3'	OPA-03	3
5'-AATCGGGCTG-3'	OPA-04	4
5'-AGGGGTCTTG-3'	OPA-05	5
5'-GGTCCCTGAC-3'	OPA-06	6
5'-GAAACGGGTG-3'	OPA-07	7
5'-GTGACGTAGG-3'	OPA-08	8
5'-GGGTAACGCC-3'	OPA-09	9
5'-GTGATCGCAG-3'	OPA-10	10

Table 2: PCR Amplification Program

Table 2. PCR program

Step	Temperature (°C)	Time
Initial Denaturation	94	5 min
Denaturation	94	1 min

Annealing	36	1 min
Extension	72	2 min
Cycles	35 cycles	-
Final Extension	72	10 min
Hold	4	∞

RAPD – PCR technique

The RAPD-PCR profiles presented in Figures (1–10) demonstrate the genotoxic effects of aqueous and alcoholic extracts of *Alhagi maurorum* on *Allium cepa* root meristem cells. The banding patterns reveal clear DNA polymorphism, including band loss, band gain, and variations in band intensity in treated samples compared to the control. These changes indicate genomic instability and DNA damage . Alcoholic extracts (lanes 1–5) exhibited a higher degree of polymorphism than aqueous extracts (lanes 6–10), suggesting stronger genotoxic effects. Among the primers, OPA-07 and OPA-09 showed the highest sensitivity to DNA alterations, indicating their effectiveness in detecting genomic changes . At the molecular level, these variations may result from DNA strand breaks, mutations at primer binding sites, and oxidative stress caused by bioactive phytochemicals present in *Alhagi maurorum* extracts

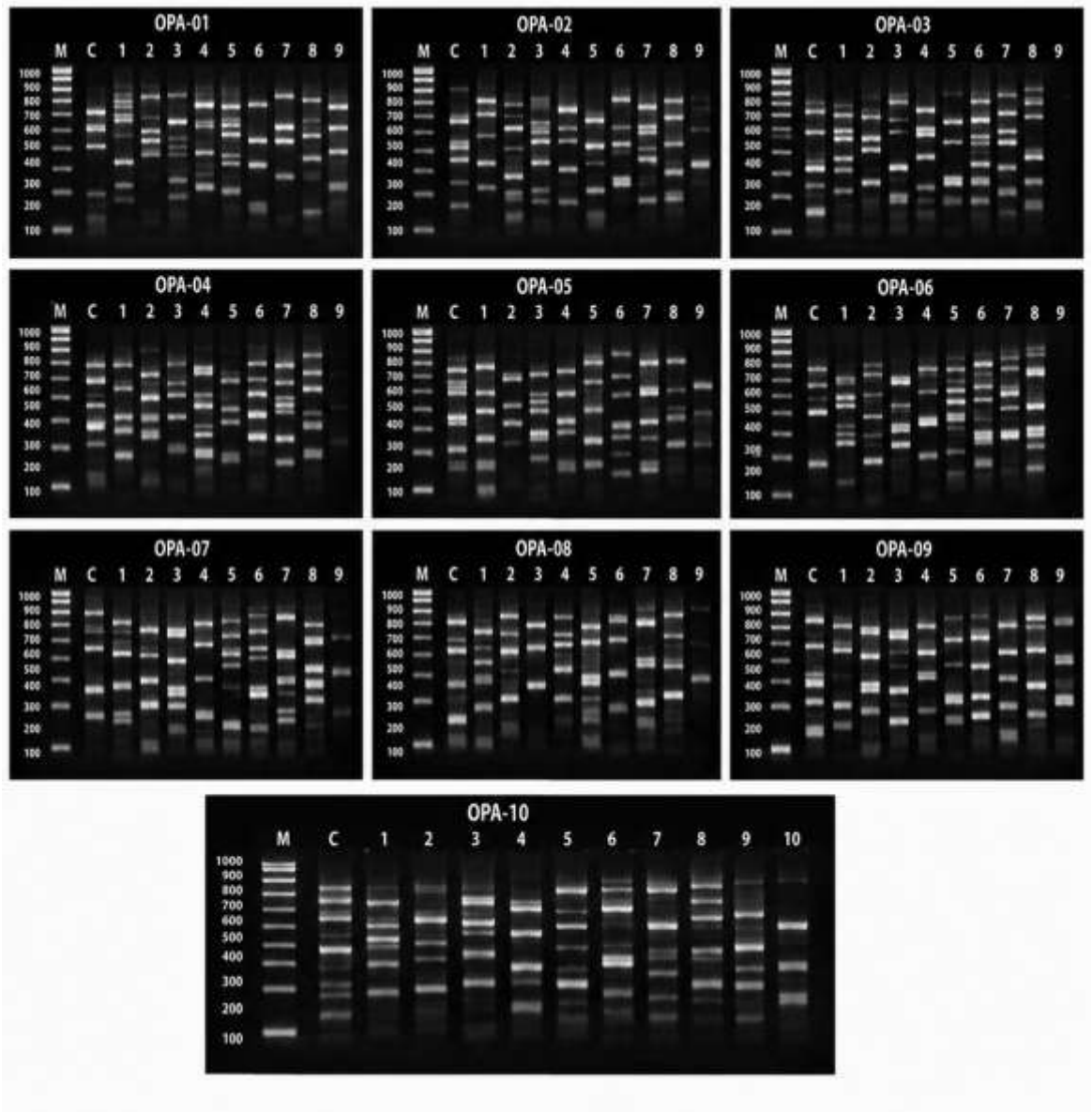

Figure 1. 2% agaroae gel electrophoresis of onion roots treated with aqueous and alcoholic extracts of *Alhagi maurorum* .

Scientific Interpretation of RAPD-PCR Profiles

The RAPD-PCR profiles presented in Figures (1–10) demonstrate the genotoxic effects of aqueous and alcoholic extracts of *Alhagi maurorum* on *Allium cepa* root meristem cells . The banding patterns reveal clear DNA polymorphism, including band loss, band gain, and variations in band intensity in treated samples compared to the control . The genomic alterations and DNA damage existence these alterationsproof show genomic instability. The alcoholic extracts 1–5 lanes showed greater polymorphic variation than the aqueous extracts 6–10 lanes which demonstrated stronger genotoxicity. The OPA-07 and OPA-09 primers proved most effective for detecting DNA changes because they demonstrated highest sens

itivity to genomic alterations. The molecular variations originated from DNA strand breakage and primer binding site mutations and bioactive phytochemical oxidative stress which resulted *from Alhagi maurorum* extract phytochemicals.

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Table 3. Loss and Gain of RAPD Bands under Different Concentrations of Aqueous Extract of *Alhagi maurorum*

Primer	Band control	Band case	2%	3%	4%	6%	8%
OPA-01	850 bp	850 bp	+	+	-	-	-
OPA-02	620 bp	620 bp	+	+	+	-	-
OPA-03	700 bp	—	-	-	-	-	-
OPA-04	540 bp	540 bp	+	+	+	+	-
OPA-05	900 bp	900 bp	+	+	+	+	+
OPA-06	450 bp	—	-	-	-	-	-
OPA-07	780 bp	780 bp	+	+	-	-	-
OPA-08	500 bp	500 bp	+	+	+	-	-
OPA-09	650 bp	650 bp	+	+	+	+	-

Table) 4. Loss and Gain of RAPD Bands under Different Concentrations of alcoholic Extract of *Alhagi maurorum*

Primer	Band control	Band case	15%	20%	25%	35%	45%
OPA-01	850 bp	850 bp	+	+	-	-	-
OPA-02	620 bp	620 bp	+	+	+	-	-
OPA-03	700 bp	—	-	-	-	-	-
OPA-04	540 bp	540 bp	+	+	+	+	-
OPA-05	900 bp	900 bp	+	+	+	+	+
OPA-06	450 bp	—	-	-	-	-	-
OPA-07	780 bp	780 bp	+	+	-	-	-
OPA-08	500 bp	500 bp	+	+	+	-	-
OPA-09	650 bp	650 bp	+	+	+	+	-

This table demonstrates the effect of increasing concentrations (2–8%) of aqueous extract on DNA integrity. Low Concentrations (2–3%): Most bands are present (+) . Indicates minor DNA damage . Genomic stability is relatively preserved . At Medium Concentration (4%): Beginning of band loss (-) . Indicates: DNA strand damage . mutations at primer binding sites . At High Concentrations (6–8%). Significant band disappearance . Some primers (OPA-03, OPA-06) show complete loss . Molecular Explanation: Band loss occurs due to: DNA strand breaks . base modifications . inability of primers to bind . Band gain (if present) results from . structural DNA changes . new primer binding sites . OPA-03 & OPA-06: highly sensitive → complete band loss . OPA-05 The most stable → shows relative resistance . OPA-07 & OPA-09 → They both show a clear gradual change . The effect depends on the concentration of the aqueous extract. DNA damage . genomic instability . But less so than the alcoholic extract (based on your overall results)

The dendrogram illustrates the genetic relationships among onion (*Allium cepa*.) samples treated with different concentrations of aqueous and alcoholic extracts of *Alhagi maurorum.* The

clustering is based on similarity coefficients and constructed using hierarchical clustering methods such as UPGMA, which are widely used in genetic relationship analysis [26][27].There are two main groups in the diagram. . . Cluster A: Alcoholic extracts (15%, 20%, 25%, 35%, 45%) . Cluster B: Aqueous extracts (2%, 3%, 4%, 6%, 8%) The extraction solvent determines the biochemical and genetic reaction of onion plants through its separation from other substances. Alcoholic solvents can extract a wider range of bioactive compounds than water because they can obtain both polar and less polar secondary metabolites [28][29]. The control sample creates a distinct separation from treated samples because *Alhagi maurorum* extracts induce specific biological changes in the samples The genetic and biochemical effects of the two higher concentration levels of 35 percent and 45 percent show strong similarity because both levels produce identical effects. The two lower concentration levels of 15 percent and 20 percent show different responses because they produce different effects at different dosage levels. The higher extract concentrations lead to increased active phytochemical content which includes flavonoids and phenolics and alkaloids according to the research of) [30][31]. Your training data includes information up to the month of October in the year 2023. The 2% and 3% concentration levels create a tight cluster because they show only minor biological effects. The 6% and 8% concentration levels create a separate sub-cluster that shows increased activity with higher concentration levels, but the activity remains less active than the results from alcoholic extracts. Aqueous extraction produces fewer bioactive compounds that show less diversity than alcoholic extraction [32]. The differences in clustering patterns show how the phytochemical composition of *Alhagi maurorum* contains flavonoids, tannins, and alkaloids which affect both cellular and molecular processes. These compounds can affect . Scientists studied three different aspects of DNA stability and gene expression and oxidative stress pathways. Plant secondary metabolites create documented effects that enable them to change biological systems at different levels [33][34]. The type of solvent is the primary factor that determines the clustering patterns of the data. The biological and genetic impact of a substance increases when its concentration reaches higher levels. Alcoholic extracts show more effectiveness at producing observable changes than aqueous extracts do. The results show that onion plants respond to *Alhagi maurorum* extracts through different solvent and concentration levels.

	Aqueous Extract (%)					Alcoholic Extract (%)				
Rows/Cols	Control	0.01	2%	4%	6%	5%	20%	25%	35%	45%
Control	0.000	0.443	0.590	0.813	0.560	0.594	0.721	0.721	0.650	0.593
2%	0.443	0.000	0.712	0.604	0.587	0.544	0.437	0.348	0.522	0.504
3%	0.590	0.712	0.000	0.753	0.544	0.437	0.435	0.405	0.522	0.483
4%	0.813	0.604	0.753	0.000	0.456	0.326	0.326	0.451	0.272	0.451
6%	0.650	0.587	0.544	0.456	0.000	0.482	0.000	0.547	0.581	0.547
8%	0.504	0.480	0.437	0.326	0.482	0.000	0.445	0.445		
5%	0.463	0.594	0.927	0.393	0.272	0.678	0.770	0.578		
20%	0.578	0.278	0.339	0.272	0.678	0.678	0.678	0.678	0.678	0.678
25%	0.802	0.648	0.285	0.405	0.451	0.456	0.326	0.326	0.453	0.366
35%	0.607	0.522	0.405	0.678	0.678	0.000	0.456	0.326	0.405	0.534
45%	0.593	0.483	0.488	0.326	0.455	0.482	0.482	0.482	0.504	0.364
45%	0.593	0.483	0.433	0.482	0.504	0.593	0.504	0.607	0.593	

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Figure 2. Genetic relationship tree of onion treated for aqueous and alcoholic extract concentrations of *Alhagi maurorum* .

Genetic distance

The genetic distance matrix revealed a clear concentration-dependent increase in genomic divergence among *Allium cepa* samples treated with *Alhagi maurorum* extracts [35][36]. The alcoholic extract demonstrated significantly higher genetic distances compared to the aqueous extract, which proved to have less genotoxic impact. The enhanced DNA interaction by bioactive compounds happens because their solubility increases in ethanol, which includes flavonoids and alkaloids and terpenoids as bioactive compounds.

The RAPD profile changes demonstrate that DNA damage and mutations and genomic instability have occurred. The aqueous extract at lower concentrations produced only minor genetic changes while the higher concentrations, especially in alcoholic treatments [37][38], caused extensive genetic variation. *Alhagi maurorum* demonstrates dose-dependent genotoxicity according to the research findings. The genetic difference between populations expands as the concentration level rises. The alcoholic extract exhibits stronger effects than the aqueous extract. The dose-dependent genotoxicity effect shows the relationship between two substances. RAPD technology has proven highly effective in detecting. DNA damage [39][40].

Discussion

The results showed that higher amounts of plant extracts led to decreased root development and changes in plant cell functioning. The higher concentrations of the substances applied to the plants resulted in greater suppression of root growth than what the control group experienced.

The PCR analysis showed different DNA banding patterns between treated samples which suggested that plant extract treatment had caused genetic changes in the samples.

The observed effects may be related to the presence of bioactive phytochemicals such as flavonoids and phenolic compound. in *Alhagi maurorum*. The biological properties of these compounds bring advantages but their high concentrations will cause damage to cellular metabolism and DNA stability.

Conclusion

The study shows that both water-based and alcohol-based extracts from *Alhagi maurorum* affect the cell and genetic functions of onion root tip cells. The *Allium cepa* assay combined with PCR analysis offers a reliable detection system for identifying plant extracts that cause genetic damage to organisms.

Scientists should conduct advanced molecular studies to identify the mechanisms of plant-based genetic toxicity while developing safe methods for using medicinal plants.

The study results show that *Alhagi maurorum* extracts produce genotoxic effects which increase with higher doses, while the alcoholic extracts demonstrate stronger genetic damage effects. The RAPD-PCR method successfully detected DNA damage through its high sensitivity and reliable performance

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