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Evaluation of the Genotoxicity and Cytotoxic Potential of GC-MS analysis of Phytoconstituents in Ethanolic Extract of *Pistacia Atlantica* on *Allium cepa L.* Root Meristem cells

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تقييم الامكانات السمية الوراثية والخلوية لتحليل GC-MS للمكونات النباتية في المستخلص الإيثانولي لنبات الفستق الأطلسي على خلايا القمم النامية لجذور نبات *Allium Cepa L.*

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Abstract:
Phenolic compounds are well-known phytochemicals found in all plants. They consist of simple phenols, benzoic and cinnamic acid, coumarins, tannins, lignins, lignans and flavonoids. Substantial developments in research focused on the extraction, identification and quantification of phenolic compounds as medicinal. Organic solvent extraction is the main method used to extract phenolics. Chemical procedures are used to detect the presence of total phenolics, and flavonoids while chromatographic techniques and spectrophotometric are utilized to identify and quantify individual phenolic compounds. This article addresses the application of different methodologies utilized in the analysis of phenolic compounds in plant including recent technical developments in the quantification of phenolics.The phytochemical investigation of ethenol extract of *P. atlantica* leaves revealed the presence of alkaloides, phenolic compounds, tannins, flavonoids, saponins, resins, glycosides. The objective of present study is to identify the flavonoids,poly phenolic in ethenol extract of *P. Atlantica* leaves using GC-MS analysis and study designed to evaluate the genotoxic and cytotoxic effects of an ethanolic extract from the Pistacia atlantica plant. The research employed the widely recognized *Allium cepa* bioassay to assess the extract's impact on mitotic division and the induction of chromosomal abnormalities in onion root tip cells. The analysis of the mitotic Index and cell division stages demonstrated a clear inhibitory effect of the *Pistacia atlantica* ethanolic extract. Treatment with both the 2 mg\ml and 20 mg\ml concentration resulted in a significant and progressive decrease in the mitotic index compared to the control group. As well as to treatment with the ethanolic extract of *Pistacia atlantica* induced a variety of chromosomal abnormalities in the Allium cepa root tips meristemal cells. The types and rates of these aberrations were found to vary depending on the concentration and exposure time.

Keywords: *Pistacia Atlantica*, Flavonoids, Phenolic, Mitotic Index, Genotoxicity.

الملخص

المركبات الفينولية هي مواد كيميائية نباتية معروفة موجودة في جميع النباتات. وهي تتكون من الفينولات البسيطة وحمض البنزويك وحمض السينامك والكومارين والتانينات واللجنين والفلافونويدات. وقد ركزت الأبحاث على استخراج المركبات الفينولية وتحديدها وقياسها كمواد طبية. يعد استخلاص المذيبات العضوية الطريقة الرئيسية المستخدمة لاستخلاص الفينولات. تُستخدم الطرق الكيميائية للكشف عن وجود الفينولات والفلافونويدات، بينما تُستخدم تقنيات الكروماتوغرافيا والطيف الضوئي لتحديد وتقدير كمية المركبات الفينولية. تتناول هذه المراجعة تطبيق الاساليب المختلفة المستخدمة في تحليل المركبات الفينولية في النباتات، بما في ذلك التطورات التقنية الحديثة في البطوم الاطلسي. كشفت الدراسة الكيميائية النباتية لمستخلص الإيثانول لأوراق النبات عن وجود قلويدات تقدير عن وجود الفينولات وتانينات وفلافونويدات وصابونينات وراتنجات وجليكوسيدات. الهدف من الدراسة الحالية هو تحديد الفلافونويدات والبوليفينول في مستخلص الإيثانول باستخدام جهاز كروماتوغرافيا الغاز -مطيافية الماس لمستخلص الايثانول, وتهدف الدراسة إلى تقييم التأثيرات السامة للجينات والسامة للخلايا للمستخلص الإيثانولي من نبات الفستق الأطلسي. حيث استخدم البحث الاختبار الحيوي *Allium cepa* المتعارف عليه بشكل واسع وذلك لتقييم تأثير المستخلص على الانقسام الانقسامي وتحريض تشوهات الكروموسومات في خلايا المرستيمية لجذور البصل. حيث أظهر تحليل المؤشر الانقسامي ومراحل انقسام الخلايا تأثيرًا مثبطًا واضحًا لمستخلص الفستق الأطلسي الإيثانولي. أدت المعاملة بتركيز 2 مجم\مل و20 مجم\مل إلى انخفاض كبير وتدريجي في المؤشر الانقسامي مقارنة بالمجموعة الضابطة. بالإضافة إلى العلاج بالمستخلص الإيثانولي لفستق الأطلسي تسبب في مجموعة متنوعة من التشوهات الكروموسومية في الخلايا المرستيمية لأطراف جذور نبات *Allium cepa*. وقد وجد أن أنواع ومعدلات هذه الانحرافات تختلف حسب التركيز ووقت التعرض.

الكلمات المفتاحية: البطوم الأطلسي، الفلافونيد، بولي فينول، معامل الانقسام، السمية الوراثية.

Introduction

The investigation into the biological activities of plant extracts is a crucial area of research, particularly as many natural compounds have a long history of use in traditional medicine [1],[2],[3]. plant possesses significant nutritional and medicinal properties which make it a good source of glucosinolates, favonoids and phenolic [4] phenolic compounds is an aromatic ring bearing one or more hydroxyl groups [5] Plant phenolic compounds are classified as simple phenols or polyphenols based on the number of phenol units in the molecule. Thus, plant phenolics comprise simple phenols, coumarins, lignins, lignans, condensed and hydrolysable tannins, phenolic acids and flavonoids [6].

Pistacia, a genus of flowering plants from the family Anacardiaceae, contains about twenty species, among them five species are more popular including P. vera, P. atlantica, P. terebinthus, P. khinjuk, and P. lentiscus [7] that are native to all of Africa, and southern Europe, warm and semi desert areas across Asia. Different parts of these species have been used in traditional medicine for various purposes like tonic, aphrodisiac, antiseptic, antihypertensive and management of dental,gastrointestinal,liver,urinary tract, and respiratory tract disorders. P. atlantica is a species of Pistacia tree known by the English common name ‘mastic’ tree and as the Persian turpentine tree. In Arabic, it is called (Butm or Butum), P. atlantica is a deciduous tree growing up to 7 m (23 ft) tall with branches spreading and growing erect to form a dense crown [8].

Genus Pistacia is characterized by the presence of a wide range of diverse compounds such as flavonoids, triterpenes, sterols and phenolic compounds. The phenolic compounds have been detected in different parts of these species, gallic acid, 3-(8-pentadecenyl) phenol; 3,4,5-tri-O-galloyl quinic acid and 1,2,3,4,6-Pentagalloyl glucose from P. vear, P. lentiscus. P. atlantica fruits [9] and the structures of these three metabolites were confirmed by spectroscopic analysis, including 1D, 2DNMR, and by negative ESIMS, HRESI mass as well. Two new ellagitannins 2,3-di-O-galloyl(α/β)-4C1-glucopyranose, nilocitin and 1,3-di-O-galloyl-β-D-4C1-glucopyranos [10].

All compounds have been characterized for the first time from leaves of Pistacia atlantica and 1,2,3,4,6-penta O-galloyl-β-D-4C_1-glucopyranose has been previously isolated from P. lentiscus fruits [11] and its extracts are of interest for their potential therapeutic properties. However, before considering any medicinal application, it is essential to rigorously evaluate the potential for adverse effects, including genotoxicity and cytotoxicity. Genotoxicity refers to the ability of a substance to damage genetic material, while cytotoxicity relates to a substance's toxic effect on cells, often leading to a reduction in cell division or cell death [1].

To assess these effects, the Allium cepa test is a well-established and reliable bioassay. It is favored for its simplicity, cost-effectiveness, and high sensitivity to a wide range of chemical and natural agents. The large, easily visible chromosomes of Allium cepa root tips meristematic cells make them an ideal model system for studying the effects of substances on mitotic division and for detecting chromosomal aberrations. The test is a standard for environmental monitoring and has been validated by numerous studies, including those by [12], [13], [14] and [15].

Material and Methods

Collection of plant material

The Fresh plant of *P. atlantica* leaves were collected from Wadi Souf Al Jin Bin Walid Libya. The plant was identifed and authenticated properly at the Herbarium of the College of Science, University of Sebha.

Preparation of powder and extract:

The leaves were left to dry in the shade at room temperature [16] and then ground into a fne powder using an electric blender Plant Extracts Preparation. Two types of plant extracts were prepared. ethnol extract was prepared by the cold maceration method as described by Ibrahim and Kebede [17]. Using this technique, 100g of dried leaf powder was added gradually to 1000mL of 80% EtOH (PanReac AppliChem, Spain) in a glass beaker under stirring and stored for 72 hours. The extract was fltered via Whatman flter paper No. 1 and dried by using a rotatory evaporator (Heiodolph, Germany) at 50°C to obtain the dried extract (residue). The residue was stored at 4°C until use. The aqueous extract was prepared by the digestion method described by [18].

Genotoxicity assessment

The Allium test was used to study the genotoxicity of chemicals and plant extracts on the chromosomes of the cells of the growing tops of the roots of the plant *Allium Cepa L.* according to Cabuga, *et al* [6] and Caritá & Marin-Morales [14], and two different concentrations (2,20 mg\ml) of the alcoholic extract were used. For a period of (12 and 24 hours), the Allium Test protocol involved examining at least three slides of each treatment was examined and the number of 1000 cells (divided and undivided) was calculated for each slide. The number of cells in each phase of mitotic division – prophase, metaphase, anaphase, and telophase was also calculated, and the percentage of cells with numerical abnormalities for each phase and the percentage of normal cells for each phase of mitotic division were determined [12],[19],[20],[21]. Then the mitotic index was determined using the following equation.

$$MI = (\text{Total number of cells} / \text{Numbers of dividing cells}) * 100$$

Statistics

All results of experiment had compared each other by one-way analysis of variance (ANOVA) with T. test. Mean values were given as Standard deviation (SD). $p < 0.05$ was used for showing significant.

Preliminary phytochemical screening

Preliminary phytochemical testing for the presence of various compounds by standard methods such as flavonoids poly phenolic, alkaloids, steroids, saponins, glycosides, terpenoids, tannins were found to be absent in the extract (Table 1).

Table 1: Results of preliminary phytochemical screening of ethanolic extract.

Phytochemical test	Name of the test	Ethanol extract
Flavonoids	Alkaline reagent test	+
polyphenolic	Ferric chloride test	+
Saponins	Frothing test	+
Alkaloids	Hager's test, Dragendroff's reagent	+
glycosides	Borntrager's test	+
Terpenoids	Salkowski's test (modified)	+
Steroids	Libermann-Burchard's test	+
Proteins and amino acids	Ninhydrin test	+
Carbohydrates Glycosides	Fehling's solutions, Molish's test	+
Tannins	Braymer's test	-

Results and discussion

Gas chromatography-Mass spectrometry (GC-MS) analysis

The extract was dissolved in EtOH and mixture of solvents and then subjected to GC/MS analysis. The sample was carried out in a PerkinElmer Clarus 600 GC System, fitted with a Rtx-5MS capillary column (30 m l, 0.25 mm) inner diameter, 0; maximum temperature 350 °C), MS. Ultra-high purity helium was used as carrier gas at a constant flow rate of 1.0 mL/min. The injection, transfer line and ion source temperatures were all 290 °C. The ionizing energy was 70 eV. Electron multiplier voltage was obtained from autotune. The oven temperature was programmed from 60 °C (hold for 2 min) to 280 °C at a rate of 3 °C/min. The crude samples were diluted with appropriate solvent (1/100, v/v) and filtered. The particle-free diluted crude extracts (1 µL) were taken ina syringe and injected into injector with a split ratio 1:30. The identification of compounds was done by comparing the spectrum of

unknown compounds with the spectrum of known compounds in their library and the name, molecular weight and structure were probably determined.

Table 2: Phytoconstituents identified in ethanol extract of *Pistacia atlantica* Desf leaves by Scan GC-MS analysis without peak area.

Peak	Retention time (min)	Name of compound	Molecular weight
1	35.91.	3-(3,4-Dihydroxyphenyl)-2-propenoic acid	176.12 g ·mol^{-1}
2	36.31	3-(3,4-Dihydroxyphenyl)-2-propenoic acid 3,4-Dihydroxycinnamic acid	180 g·mol^{-1}
3	40.18	(2E)-3-(4-hydroxy-3-methoxyphenyl) prop-2-enoic acid	194.186 g·mol^{-1}
4	42.45	5,7-Dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one	274.240 g·mol^{-1}
5	45.79	(2R,3S)-2-(3,4-Dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol Ferulic acid	290.271 g·mol^{-1}
6	48.19	(2E)-3-(4-gluco-3-methoxyphenyl) prop-2-enoic acid	386 g·mol^{-1}
7	52.20	2-(3-hydroxy,4-glucophenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one	405 g·mol^{-1}
8	54.74	3,5-Dimetoxy-2-(4-methoxyphenyl)-7- (2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl] oxy}-4H-1-benzopyran-4-one	500 g·mol^{-1}
9	57.26	3-(3,4-glucophenyl)-2-methylepropenoic acid	520 g·mol^{-1}
10	59.01	(2R,3S)-2-(3,4-Diglucophenyl)-3,4-dihydro-2H-chromene-3,5,7-triol Ferulic acid	616 g·mol^{-1}

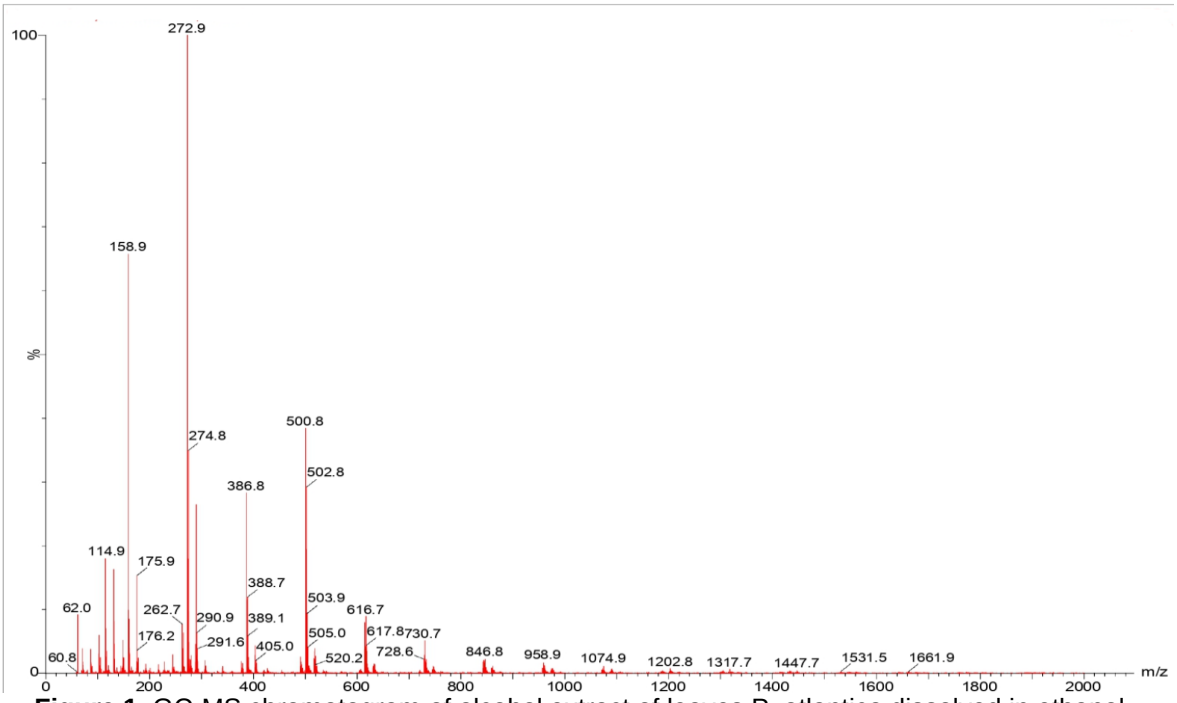

Figure 1: GC-MS chromatogram of alcohol extract of leaves P. atlantica dissolved in ethanol

Cytotoxic Effects on Mitotic Activity

effects of the ethanol extract on the cleavage coefficient and the different cleavage stages:

The analysis of the mitotic index and cell division stage demonstrated a clear inhibitory effect of the *Pistacia atlantica* ethanolic extract. As shown in Table 3, treatment with both the 2mg\ml and 20mg\ml concentrations resulted in a significant and progressive decrease in mitotic index compared to the control group.

Table 3: Effect of ethanolic extract on the cleavage coefficient and cleavage stages when treated at different concentrations for 12 and 24 hours.

Time (hours)	Con.(mg\ml)	Total Cells (X±SD)	Mitotic Index (MI) (X±SD)	الخلايا المنقسمة (X±SD)	Prophase (X±SD)	Metaphase (X±SD)	Ana - Telophase (X±SD)
12	Control	1390.00 ± 138.83	39.390± 5.550	±40.2782 543.333	±56.4476 413.667	29.67 ± 4.163	100.00 ± 32.047
	2	2044.67 ± 304.560	22.853 ± 2.338	±27.0247 462.667	±17.7858 310.667	19.33 ± 10.599	132.67 ± 12.90
	20	2061.00 ± 128.00	11.560 ± 1.061	±32.9242 239.000	±30.5123 235.000	0.00 ± 0.000	4.00 ± 5.20
	p-value	0.191**	0.191**	0.00	0.016*	0.016	0.00
24	Control	1390.00 ± 138.83	39.390± 5.550	±40.2782 543.333	±56.4476 413.667	29.67 ± 4.163	100.00 ± 32.047
	2	2083.33 ± 642.22	25.257 ± 7.192	±74.8955 500.333	±71.7101 338.333	338.33 ± 71.710	14.33 ± 7.572
	20	2324.33 ± 658.160	15.387 ± 2.009	±51.4684 349.000	±49.8698 348.000	348.00 ± 49.870	0.00 ± 0.00
	p-value	0.191**	0.191**	0.00	0.016*	0.016	0.00

The Mitotic Index showed a dose-dependent reduction, with the 20 mg\ml concentration causing a more pronounced decrease than the 2 mg\ml concentration. A deeper analysis of the data, however, reveals a more complex relationship between the extract's effect and exposure duration. While one would typically expect a continued decline in MI over time with a toxic substance, the mitotic index values were slightly higher at 24 hours compared to 12 hours for both concentrations. For example, the MI at 2 mg\ml increased from 22.85 at 12 hours to 25.25 at 24 hours. This finding Suggest a nuanced biological response, which may not be a simple linear dose-response relationship. It is possible that the initial 12 hours. Exposure caused an acute stress on the cells, leading to a severe cell cycle arrest. Over the following 12 hours, a subset of the cells might have adapted or recovered from the stress, allowing them to re-enter the mitotic cycle, which would account for the slight increase in MI at 24-hour time point.

Further examination of the statistical data in Table 3 reveals an important detail. The p-value for the Mitotic Index was 0.191, which is above the 0.05 significance level, indicating that the overall change in the MI itself was not statistically significant. This appears to be in contrast to the discussion, which notes a "significant decrease". However, the p-values for the number of divided cells and the individual mitotic phases (Prophase, Metaphase, Anaphase, and Telophase) were all 0.00 or 0.016, which is highly significant. This indicates that while the overall ratio of dividing cells (the MI) may have been subject to variability, the extract's effect was a statistically significant and profound disruption of the cell cycle itself, leading to a redistribution of cells among the different mitotic phase. The extract's primary effect is not a simple reduction in the mitotic rate but a specific and significant blockage of cell cycle progression.

The findings from this study are consistent with and build upon previous research into the biological activity of Pistacia extracts and other plant compounds. The observed dose-dependent increase in cytotoxicity as measured by the reduction in the mitotic index and the number of dividing cells, aligns with the findings of Al-Qubbi and Zurad [1]. Their study, which also used the Allium cepa test, concluded that increasing the concentration of Pistacia extract leads to a proportional increase in cytotoxic effects.

The statistical data reveals a particularly important distinction. While the overall mitotic index showed high variability and was not statistically significant, the profound and significant changes in the number of divided cells and their distribution across the mitotic phase indicate that the extract's primary effect is not a general reduction in cell division but a targeted disruption of key cell cycle checkpoints. The extract's ability to act as a spindle poison, as evidenced by the complete metaphase blockage at 20 mg\ml, is the most likely cause of this severe disruption.

Types of chromosomal abnormalities caused by the ethanol extract of the *Pistacia atlantica* plant on the root tips of the *Allium cepa L.*:

Treatment with the ethanolic extract of P.atlantica induced a variety of chromosomal abnormalities in the Allium cepa root meristematic cells. The types and rates of these aberrations were found to vary depending on the concentration and exposure time.

Prophase and Metaphase Abnormalities

The data in Table 4-a indicates that the highest rate of chromosomal abnormalities occurred during the prophase stage at the 2mg\ml concentration. The most common aberrations observed in this phase were irregular, granular, and Spindale Disturbance. As shown in Figure 2.

A particularly significant finding wae the complete absence of the metaphase stage when cells were treated with the 20mg\ml concentration for both 12 and 24 hours. The accompanying discussion attributes this phenomenon to the “non-formation of spindle fibers”. This observation is supported by the aberrations seen at the lower concentration, where “colchicine-like chromosome” and lagging chromosomes were the most significant abnormalities. Colchicin is a well-know antimitotic agent that inhibits the polymerization of Tubulin.

The primary component of microtubules, thereby preventing the formation of the mitotic spindle.The observation of similar effects in the treated cells strongly suggests that the *P.atlantica* extract contains compounds that function as spindle poisons, which disrupt the normal alignment of chromosomes on metaphase plate and subsequently block the cell cycle at this critical checkpoint. This explains the dose-dependent inhibitory effect on the mitotic index and the complete absence of metaphase at the highest concentration. aligns with the findings of Al-Qubbi and Zurad [1], and Arrish and Al-janga [22].

Table 4-A: Types of chromosomal abnormalities in the cells of the growing tops of *Allium cepa L*. roots after treatment with different concentrations of ethanolic extract.

Time (hours)	Con. (mg\ml)	Prophase Abnormalities					Metaphase Abnormalities		
		Abnormal (X±SD)	Irregular (X±SD)	Granular (X±SD)	Sticky (X±SD)	Spindale Disturbance (X±SD)	Colchicine (X±SD)	Lagging Chromosome X±SD	Abnormal X±SD
12	Control	0.00±0.000	0.667±1.155	0.333±0.560	0.00±0.000	0.00±0.000	1.00±1.000	0.00±0.000	0.00±0.000
	2	1.00±1.732	32.33±14.36	3.00±5.196	19.00±5.000	6.33±2.887	2.33±2.517	2.00±1.732	3.67±4.042
	20	0.00±0.000	43.67±18.18	34.33±5.508	0.00±0.000	0.00±0.000	0.00±0.000	0.00±0.000	0.00±0.000
	p-value	0.00	0.00	0.00	0.00	0.00	*0.032	*0.032	0.00
24	Control	0.00±0.000	0.667±1.155	0.333±0.577	0.00±0.000	0.00±0.000	1.00±1.000	0.00±0.000	0.00±0.000
	2	50.00±27.40	6.333±10.97	0.67±1.155	39.33±32.66	8.67±5.686	2.67±1.155	2.33±2.082	1.67±1.155
	20	69.00±52.68	91.00±54.67	0.67±1.155	0.00±0.000	0.00±0.000	0.00±0.000	0.00±0.000	0.00±0.000
	p-value	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Anaphase and Telophase Abnormalities

As shown in Table 4-b, the most significant chromosomal abnormalities observed during the anaphase and Telophase stages were bridges, lagging chromosomes, and fragments. As shown in figure 2. The presence of these aberrations provides further insight into the extract’s mechanism of action. Chromosomal bridges are often indicative of DNA breaks that have been incorrectly repaired or fused, while fragments are pieces of chromosomes that have been left behind during anaphase due to breakage. Lagging chromosomes can be caused by either improper spindle function or damage to the kinetchores, which are the protein structures on chromosomes where the spindle fibers attach.

The coexistence of both spindle-related effects (the complete blockage of metaphase and colchicine-like chromsomes) and DNA damage-related effects (bridges and fragments) indicates that the ethanolic extract of P. atlantica operates through a multi-modal mechanism of action.It dose not simply disrupt one aspect of the cell cycle; rather, it appears to simultaneously interfere with the mitotic spindle apparatus and induce structural damage to the chromosomes themselves. This dual activity accounts for the observed broad range of cytotoxic and genotoxic effects

The appearance of specific chromosomal abnormalities, such as bridges and lagging chromosomes, also corroborates the work of Arrish and Al-janga [22] and Al-Qubbi and Zurad [1], who demonstrated that the rate of such aberrations increases with both the concentration and duration of treatment. The present study’s results show a similar trend, where the rate of abnormalities increased from 12 to 24 hours at the lower concentration.

This is a critical finding, as it points to a specific molecular mechanism of action rather than a non-specific toxic effect. The co-occurrence of other aberrations, such as chromosomal bridges and fragments, indicates that extract’s compounds likely possess multiple mechanisms of action, simultaneously targeting the mitotic spindle and causing direct damage to DNA or chromosome structure. This multi-modal activity makes the extract a compelling subject for further study.

Table 4-B: Types of chromosomal abnormalities in the cells of the growing tops of *Allium cepa L*. roots after treatment with different concentrations of ethanolic extract.

Time (hours)	Con. (mg\ml)	Anaphase and Telophase Abnormalities						
		Sticky (X±SD)	Abnormal (X±SD)	Bridges (X±SD)	Breaks (X±SD)	Lagging Chromosome (X±SD)	Fragment (X±SD)	Unequal Chromosome Count (X±SD)
12	Control	0.33±0.577	0.00±0.000	0.00±0.000	0.00±0.000	0.33±0.577	0.00±0.000	0.00±0.000
	2	0.00±0.000	0.33±0.577	2.333±2.082	0.67±1.155	1.33±1.528	0.67±1.155	0.67±1.155
	20	3.67±4.619	0.00±0.000	0.33±0.577	0.00±0.000	0.00±0.000	0.00±0.000	0.00±0.000
	p-value	0.132	0.132	0.132	0.132	0.132	0.132	0.132
24	Control	0.33±0.577	0.00±0.000	0.00±0.000	0.00±0.000	0.33±0.577	0.00±0.000	0.00±0.000
	2	0.00±0.000	0.33±0.577	1.67±0.577	1.00±1.000	2.33±4.042	0.00±0.000	1.33±1.528
	20	0.67±1.155	0.00±0.000	0.33±0.577	0.00±0.000	0.00±0.000	0.00±0.000	0.00±0.000
	p-value	0.539	0.539	0.539	0.539	0.539	0.539	0.539

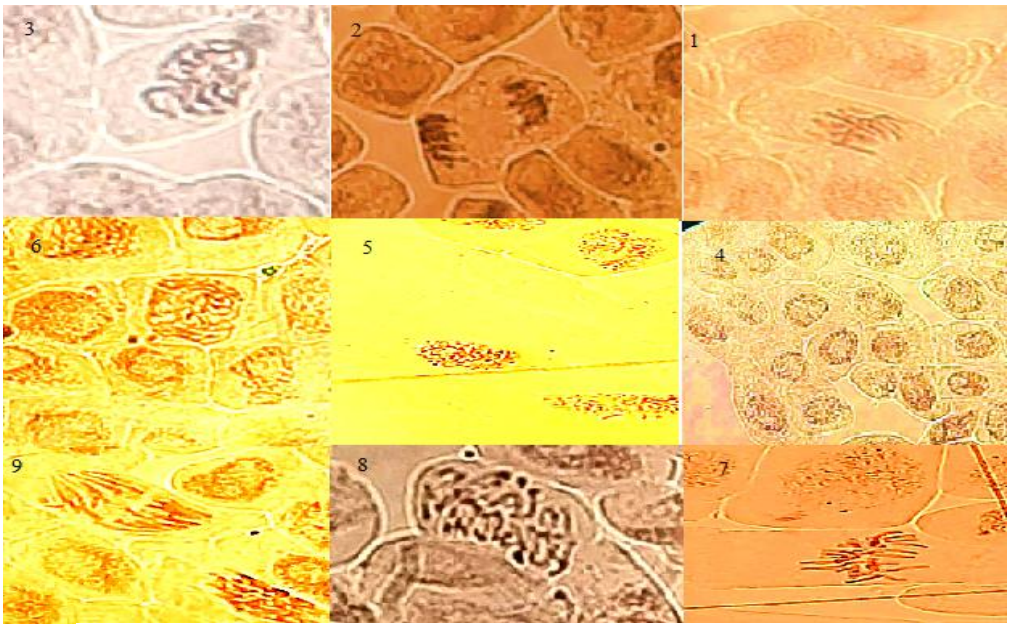

Figure 2: The stages of mitotic division and some types of chromosomes abnormalities in root cells of Allium cepa L. (1). Normal metaphase, (2). Normal Telophase, (3). Normal prophase, (4 &5). Granular prophase, (6). Spindale disturbance at prophase, fragment chromosomal and lagging chromosome, (7). Abnormal metaphase, (8). Colchicine metaphase, (9). Breaked bridge at anaphase, fragment chromosomes and chromosome loss.

Conclusion

Based on the evidence from the Allium cepa bioassay, the ethanolic extract of Pistacia atlantica possesses significant anti-mitotic and genotoxic properties. The extract's effects are both dose- and time-dependent, resulting in a substantial reduction in cell division and the induction of a wide range of chromosomal aberrations. The observed effects, particularly with the presence of colchicine-metaphase, strongly suggest that the extract contains compounds that act as spindle poisons. The additional presence of chromosomal bridges and fragments indicates a second, independent mechanism of action, likely involving direct chromosome damage. This multi-model activity makes the extract a compelling subject for further study. To fully understand and potentially harness these effects, it is recommended that future research focus on the following areas:

- Chemical Characterization: The active compounds responsible for the observed Cytotoxic and genotoxic effects should be isolated and chemically characterized. This would allow for a more precise understanding of the specific molecules driving the anti-mitotic activity.
- Expanded Dose-Response Analysis: Conducting a study with a wider range of concentrations and time points would provide a more complete picture of the dose-response relationship and the kinetics of the extract's effects.
- Cross-species Testing: The genotoxic and Cytotoxic effects of the isolated compounds should be evaluated in other model systems, such as Human Cancer cell lines. This would be a Crucial step toward assessing the potential of these compounds as natural chemotherapeutic agents, given their potent anti-mitotic activity.

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