ا لمجهروية الجزائرية ادليمقريطاة اليبعشة
People's Democratic Republic of Algeria
وزارة العتليم العطاي والثحب العلمي
Ministry of Higher Education and Scientific Research

Ibn Khaldoun University, Tiaret
Faculty of Natural and Life Sciences
Department of Biology

Dissertation

Submitted in partial fulfilment of the
requirements for the degree of

Master of Biological Sciences

Field: **Natural and Life Sciences**
Branch: **Biological Sciences**
Speciality: **Cell and Molecular Biology**

Presented by:

Abir Amina GHARBI
Hadjer Fatima Zohra HANNACHE

Title

Study of cytogenotoxicity of Prickly Juniper *Juniperus oxycedrus* subsp *oxycedrus* L.

Jury members:

President	Mr. Toufik BENAISSA	University of Tiaret
Examiner	Dr. Abdelkader TADJ	University of Tiaret
Supervisor	Prof. Khaled TAIBI	University of Tiaret
Co-supervisor	Dr. Kada SOUANA	University of Tiaret

July 2024

الجمهورية الجزائرية الديمقراطية الشعبية
République Algérienne Démocratique et Populaire
وزارة التعليم العالي والبحث العلمي
Ministère de l'Enseignement Supérieur et de la Recherche Scientifique

Université Ibn Khaldoun, Tiaret
Faculté des Sciences de la Nature et de la Vie
Département de biologie

Mémoire

Présenté en vue de l'obtention du diplôme de

Master académique

en

Domaine : **Sciences de la Nature et de la Vie.**
Filière : **Sciences Biologiques.**
Spécialité : **Biologie Moléculaire et Cellulaire.**

Présenté par :

Abir Amina GHARBI
Hadjer Fatima Zohra HANNACHE

Intitulé

Étude de la cytogénotoxicité du Genévrier oxycèdre *Juniperus oxycedrus* subsp. *oxycedrus* L.

Soutenu publiquement le :

Devant les membres de jury :

Président	M. Toufik BENAISSA	Université de Tiaret
Examinateur	Dr. Abdelkader TADJ	Université de Tiaret
Encadrant	Prof. Khaled TAIBI	Université de Tiaret
Co-encadrant	Dr. Kada SOUANA	Université de Tiaret

Année universitaire 2023-2024

Acknowledgement

First and for most, we express our gratitude to Allah for enabling us to complete this work

We are deeply grateful to all those who have assisted and supported us, and we extend our sincerest appreciation to Mr. BENAISSA. T, whose exceptional leadership allowed us the privilege of serving as jury president despite his numerous commitments.

We would like to extend our sincere gratitude to Prof. Khaled TAIBI for his valuable guidance, availability, and supervision throughout the completion of this work.

This work would not be as rich and could not have had the day without the help of Mme. Khadija MEKNASSI we thank her for the quality of his exceptional supervision, for his patience, his rigor and availability during our preparation of this brief.

I would like to express my sincere appreciation to Mme. Fatima Zohra SAMMAR for her tireless assistance and dedication in the laboratory.

Our thanks also go to all the teachers, especially Prof. Leila AIT ABDERRAHIM Leila, Dr SOUANA, and Dr. TADJ and prof. Mohamed boussaid

Our thanks also go to Dr. TADJ for accepting to be in this jury. I would like to extend my gratitude to my friends and all those who have contributed, either directly or indirectly, to the development of this work.

Dedication

To my beloved parents, who continually provided their love and support both emotionally and financially.

To my only brother MOHAMED and sisters ASMA, HIBA, HOUDA who have been a source of inspiration.

To my dearest friend AFFAF, for keeping me motivated and for always believing in me.

To my dear partner ABIR, my guiding light, thank you for being my biggest motivator.

This thesis would not have been possible without the unwavering support of our friends, everyone who believed in me, my second family INNOVATION CLUB thanks for being a source of inspiration.

Lastly to me, for the long nights of research, the moments of self-doubt, and the sheer perseverance required to reach this milestone. This achievement is a reflection of your unwavering commitment to growth.

Hadjer

Dedication

To my mother, who supported me tirelessly and sacrificed so much to see me succeed, and to my father, whose unwavering belief in me has been a constant source of strength.

To my family, whose love and encouragement have been my foundation.

This work is also dedicated to all the friends and mentors who have guided and helped me throughout this journey.

I also dedicate this work to my research partner, who shared this effort with me and helped me along the way and gave me more energy to carry on.

Thank you for your endless support and inspiration.

To a special person, Assil, who supported and stood by me quietly and steadfastly throughout this journey. Your encouragement and assistance have been invaluable, and I am deeply grateful for your unwavering support. Thank you for believing in me and for being my silent strength.

Abir

List of figures

Figure 1. Aspect of *J. oxycedrus* L. (© 2024). 2
Figure 2. Needles of *J. oxycedrus* L. (© 2024). 3
Figure 3. Global presence of *J. oxycedrus* L. 4
Figure 4. The most common testing methods for the assessment of genotoxic substances. . 9
Figure 5. Steps of material plant preparation. 11
Figure 6. Aqueous extract. 12
Figure 7. Ethanolic extract. 12
Figure 8. Procedures of *A. cepa* test. 14
Figure 9. Variation of total phenolic contents in the aqueous and ethanolic extracts of *J. oxycedrus* L. 16
Figure 10. Flavonoids content of the tested plant extracts. 17
Figure 11. Tannins content for the tested plant extracts. 18
Figure 12. 4. Variation of antioxidant activity (DPPH) for the tested plant extracts of *J. oxycedrus* L. 18
Figure 13. Variation of root number of *A. cepa* under the effect of *J. oxycedrus* L. extracts. 19
Figure 14. Variation of root length of *A. cepa* under the effect of *J. oxycedrus* L. extracts . 20
Figure 15. Variation of root diameter of *A. cepa* under the effect of *J. oxycedrus* L. extracts. 20
Figure 16. The effect of *J. oxycedrus* extracts on mitotic index (MI) and mitotic phases in the root' cells of *A. cepa*. 21
Figure 17. The effect of *J. oxycedrus* extracts on prophase in the root cells of *A. cepa*. 22
Figure 18. Normal prophase (© 2024). 22
Figure 19. The effect of *J. oxycedrus* extracts on metaphase in the root cells of *A. cepa*... . 23
Figure 20. Normal metaphase (© 2024). 23
Figure 21. The effect of *J. oxycedrus* extracts on anaphase in the root cells of *A. cepa*..... 24
Figure 22. 14.Normal anaphase (© 2024). 24
Figure 23. The effect of *J. oxycedrus* extracts on telophase in the root cells of *A. cepa*. 25
Figure 24. Normal telophase (© 2024). 25
Figure 25. Chromosomal aberrations observed in *A. cepa* treated with *J. oxycedrus* extracts (© 2024). 27

List of tables

Tableau 1. Yield of the plant extracts .....

Tableau 2. Chromosomal aberrations of *Allium cepa* cells treated with *J. oxycedrus* Extracts.....

List of abbreviations

$AlCl_3$:	Aluminum chloride
AP:	Advanced placement
BER:	Base excision repair
CAs:	Chromosomal aberrations
DNA	Deoxyribonucleic acid
DPPH:	2,2-diphenyl-1-picrylhydrazyl
DSB:	Double strand break
DSBR:	Defense science board report
GAE/mL:	Gallic acid equivalent per milliliter
GBM:	Glioblastoma
GG- NER:	Global genome nucleotide excision repair
HCC:	Hepatocellular Carcinoma
HCL:	Hydrochloric acid
HR:	Homologous recombination
MI:	Mitotic index
MMR:	Mismatch Repair
MMS:	Methyl methanesulfonate
NER:	Nucleotide excision repair
NHEJ:	Non-homologous end-joining
NOR:	The nucleolus organizer region
PI:	Phase Index
PI:	Phase Index
QE/mL:	Quercetin equivalent per milliliter
RNA:	Ribonucleic acid
SD:	Standard deviation
TAE/mL:	Tannic acid equivalent per milliliter
TAE/mL:	Tannic acid equivalent per milliliter
TC-NER:	Transcription coupled nucleotide excision repair
UV:	Ultraviolet

Table of contents

ملخص .....
Abstract.....
Resumé .....
- List of figures .....
- List of abbreviations.....
- Table of contents .....

State of the Art

1. *Juniperus oxycedrus* 2
2. cytotoxicity 7
3. Genotoxicity..... 8
4. *Allium cepa* assay..... 10

Methodology

1. Preparation of plant extracts..... 11
2. Phytochemical compounds..... 13
2.1. Polyphenols 13
2.2. Flavonoids 13
2.3. Tannins 13
3. Antioxidant activity using DPPH radicals scavenging assay 13
4. Mitotic index analysis..... 13

Results

1. Evaluation of the yield of extraction 16
2. Evaluation of the phytochemical compounds..... 16
2.1. Total phenolics content..... 16

2.2. Total flavonoids content 17
2.3. Total tannins content 17
3. Evaluation of the antioxidant activity 18
3.1. DPPH 18
4. Evaluation of the cytotoxicity 19
4.1. Root morphometry 19
4.2. Cell phases 21
5. Evaluation of the genotoxicity 26

- Discussion 29

- Conclusion 32

- References 33

Introduction

Introduction

For centuries, medicinal plants have been used in traditional medicine practices for their healing properties. According to the World Health Organization (WHO, 2013), it is estimated that approximately 80% of the global population relies on botanical preparations as medicines to address their health needs. The use of medicinal plants is a fundamental and integral part of traditional medicine practices worldwide, playing a significant role in the healthcare systems of many cultures (Taïbi et al. 2021). They contain bioactive compounds that have been shown to exhibit various medicinal properties, including antibacterial, antifungal, anti-inflammatory, antioxidant, and anticancer effects among many others (Benzie et al. 2018).

Prickly juniper (*Juniperus oxycedrus* L.) is a medicinal plant with a long history of use in traditional medicine (Taïbi et al. 2020). It has been extensively studied for its biological activities and pharmacological properties (Kumar et al, 2017). The aqueous and ethanolic extracts of prickly juniper have been investigated for their potential therapeutic applications (Patel et al. 2018). The aqueous extract has been demonstrated to exhibit robust antioxidant and free radical scavenging capabilities (El-Hawary et al. 2019) which are responsible in turn of high anti-inflammatory potential (Patel et al. 2018). Additionally, the antimicrobial activity of this species was also demonstrated against a diverse range of microorganisms (Ahmed et al. 2018) highlighting its potential as a therapeutic agent against infections. In contrast, the ethanolic extract of *J. oxycedrus* has been extensively investigated for its cytotoxic potential and anti-cancer activities (Ahmed et al. 2018). Ali et al. (2019) have reported its potent cytotoxic effects against human cancer cell lines.

The primary goal of this research is to valorise Algeria's natural medicinal plant resources from a part, and the Algerian pharmacopeia from another part, focusing on the collection and compilation of comprehensive information to enhance our understanding about the use of medicinal flora and its potential applications. The specific aim of the present study is to evaluate the cytotoxicity and genotoxicity of aqueous and ethanolic extracts of Algerian Prickly juniper *J. oxycedrus* subsp *oxycedrus* L. in order to determine the level of its toxicity and the legitimacy of its use in traditional and/or modern pharmacology.

Literature review

1. Description of prickly juniper

The prickly juniper (*Juniperus oxycedrus* L.) is a compact shrub or small tree, reaching a height of 3-5 meters. It is a member of the Cupressaceae family, also commonly referred as the sharp cedar or cade juniper. In its early stages, the tree's crown exhibits a conical shape, whereas in maturity, it assumes an irregular outline. The trunk's bark is characterized by fibrous grey to brown-red stripes, which peel off in longitudinal strips. The tree is distinguished by its numerous branches, which spread or ascend in a sprawling manner (Vilar et al. 2016).

Figure 1. Aspect of *Juniperus oxycedrus* L. (© 2024).

The leaves of the prickly juniper are needle-like and arranged in whorls of three, alternating along the stem. Each needle is 1-2.5 cm in length and 1-2.5 mm in width, with two shallow, waxy furrows containing stomata on the upper surface. The lower surface features a prominent ridge, while the tip is tipped with a spiny projection (Vilar et al. 2016).

Figure 2. Needles of *Juniperus oxycedrus* L. (© 2024).

The male cones of the prickly juniper are small, rounded, and yellow in color. In contrast, the female cones are larger, bluish-green, and rounded to oblong in shape. The fruit produced by the female cones is a small, dark blue-purple berry with a fleshy texture. Each berry typically contains one to three seeds (Arli et al. 2016).

2. Taxonomy

According to the Angiosperm Phylogeny Group (APG 2016), Prickly juniper is classified as a member of the family Cupressaceae. The sort of this plant is *Juniperus*, while the specific species is *Juniperus oxycedrus* L. In Arabic, this plant is referred to as Âarâar or Tagga. In French, the popular names for this plant include cade oxycèdre, genévrier oxycèdre, or cadier. In English, it is commonly known as medlar tree, juniper bush, prickly cedar, sharp cedar or juniper berry.

3. Geographical distribution

The juniper species are native to the Mediterranean region, spanning from Morocco and Portugal to Lebanon and Syria, and extending to Kurdistan in Iran, Iraq, and the Caucasus mountains. There are four recognized subspecies (i) *Juniperus oxycedrus* subsp. *oxycedrus* found in both inland and coastal areas, (ii) *Juniperus oxycedrus* subsp. *macrocarpa* common along the Mediterranean and Black Sea coasts, (iii) *Juniperus oxycedrus* subsp. *badia* found in inland

regions of North Algeria and the Iberian Peninsula, and (iv) *Juniperus oxycedrus* subsp. *transtagana* which inhabits lowlands and coasts in central Portugal and southwestern Spain (Vilar et al. 2016).

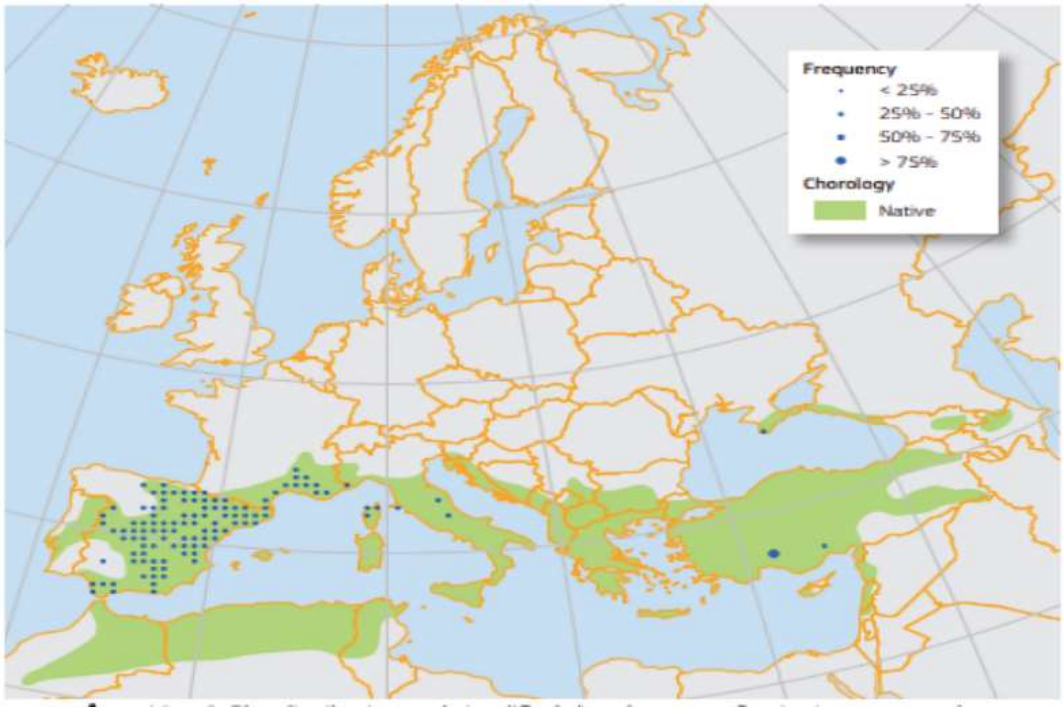

Figure 3. Global presence of *Juniperus oxycedrus* L.

In Algeria, *Juniperus oxycedrus* is found in various bioclimatic zones, including humid and hyper-arid regions. According to the Flora of Algeria, this species inhabits the mountainous areas of the country, including the Atlas Mountains, Aurès Mountains, and Kabylie Mountains. Additionally, it has also been reported in the semi-arid and sub-humid climates of the Northeastern High Plateaus. (Kacem et al. 2010).

4. Phytochemistry

Juniperus oxycedrus is rich in flavonoids, flavones, terpenoids, monoterpenoids, and sesquiterpenoids. These compounds are found in the plant's leaves, infructescence, and fruits. The leaves also contain terpenoids, monoterpenoids, and fatty acids, including sabinic acid. Fatty acids such as palmitic, linoleic, and linolenic acid have been isolated from the plant (Ait-Ouhammou et al , 2018)

Secondary metabolites are a diverse group of plant chemicals that are not essential for plant growth, but play crucial ecological roles as defense mechanisms against herbivores, pathogens, or competitors (Macheix et al. 2005). These metabolites exhibit varying distribution patterns, chemical classes, and functions, and often possess complex structures that can accumulate in specific tissues or organs. As a result, they have become valuable sources of bioactive

compounds with potential applications in various fields, such as pharmaceuticals, fragrances, and food additives (Yinping Lei, 2017).

4.1. Polyphenols

Polyphenols are a type of plant compound characterized by the presence of multiple phenolic groups. These molecules have antioxidant and anti-inflammatory properties, which contribute to their potential health benefits (Kähkönen et al, 2008). Polyphenols are found in a wide range of plant-based foods, including fruits, vegetables, grains, and *Juniperus oxycedrus* leaves. They have been linked to reduced risk of chronic diseases such as heart disease, cancer, and cognitive decline (Haddouchi et al, 2016). Additionally, polyphenols may help to improve cardiovascular health by improving blood flow and reducing blood pressure. Overall, the consumption of polyphenol-rich foods is recommended as part of a healthy diet for overall well-being (Urquiaga and Leighton 2000).

4.2. Flavonoids

Flavonoids are a subclass of polyphenolic compounds that have been shown to have numerous health benefits. Flavonoids may help to improve cardiovascular health by reducing inflammation and improving blood vessel function (Rodriguez-Mateos et al. 2018). Additionally, flavonoids have been found to have antioxidant and anti-cancer properties, and may help to protect against age-related cognitive decline (Wang et al. 2017). Flavonoids are found in a variety of fruits, vegetables, and beverages, including apples, berries, tea, and chocolate. They are an important part of a healthy diet and may help to reduce the risk of chronic diseases (Tsimogiannins, 2006).

4.3. Tannins

Tannins are a type of polyphenolic compound found in plants, particularly in the bark, seeds, and stems of fruits, and leaves (Scalbert et al, 1991). They are known for their astringent and bitter taste, and are often responsible for the dryness or puckering sensation felt in the mouth when consuming certain foods and drinks (LaCroix et al, 2002). Tannins have been shown to have antioxidant and anti-inflammatory properties, and may help to protect against chronic diseases such as heart disease and certain types of cancer (Delić et al. 2018).

5. Biological activities

5.1. Antimicrobial activity

Juniperus oxycedrus essential oils showed antimicrobial activity against 16 bacterial isolates namely *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus subtilis, Bacteroides fragilis, Clostridium perfringens, Enterococcus faecalis, Haemophilus influenzae, Moraxella catarrhalis, Neisseria gonorrhoeae, Streptococcus agalactiae, Streptococcus pneumoniae,* with *Haemophilus influenzae* being the most sensitive (Al-Dabbas et al. 2015). The plant's leaves and fruits also exhibited antimicrobial activity against various bacterial and fungal species. The methanolic extract of *J. oxycedrus* leaves showed inhibition zones against several microorganisms, including *Bacillus subtilis, Staphylococcus aureus,* and *Candida albicans* (Al-Dabbas et al. 2015).

5.2. Antioxidant activity

The antioxidant capacity of *J. oxycedrus* was studied using different *in vitro* assays. The ripe "berries" methanol extracts showed higher antioxidant activity than the wood extracts. The aqueous extract exhibited high antioxidant activity as measured by DPPH, TEAC, and FRAP assays (Tang et al, 2015)

5.3. Hypoglycemic activity

The hypoglycemic and antidiabetic activities of *J. oxycedrus* were evaluated using normal, glucose-hyperglycemic, and streptozotocin-induced diabetic rats. The non-polar fraction of the n-hexane sub extract showed antidiabetic activity and was found to contain fatty acids such as palmitic, linoleic, and linolenic acid (Cicek et al. 2015).

5.4. Analgesic and anti-inflammatory activities

Methanol and dichloromethanol extracts of *J. oxycedrus* leaves and stems exhibited analgesic and anti-inflammatory effects in various models. The methanol extract showed significant effects on chemical stimulus tests, while the dichloromethanol extract only showed significant activity on mechanical stimulation. Both extracts at doses of 200 mg/kg caused a significant decrease in motor activity. (Moreno et al. 2015).

6. Traditional uses

The berries of *Juniperus oxycedrus* subsp. *oxycedrus* L. have been traditionally used in Turkey as a decoction to lower blood glucose levels, consumed as tea or pounded fruits (Orhan et al. 2012). The cade oil extracted from this plant has been employed extensively in various

applications, with the most significant usage being as a fragrance component in soaps, detergents, creams, lotions, and perfumes. Additionally, Cade oil has been widely used in traditional medicine to treat a range of conditions, including chronic eczema, psoriasis, and other skin diseases, as well as infectious diseases such as tuberculosis, bronchitis, and pneumonia (Al-Snafi and Ali 2018).

7. Cytotoxicity

The adverse impact of toxic substances on cells is referred to as cytotoxicity. This phenomenon occurs when toxic agents harm or destroy cells, leading to cell death, damage, or malfunction (Erman et al. 2019). Understanding cytotoxicity is essential in the fields of toxicology and biocompatibility testing, as it enables the evaluation of the potential risks associated with exposure to toxic substances (Erman et al. 2019).

Cytotoxic agents are substances that are toxic to cells, encompassing factors that prevent cell growth and sometimes cause cell death (Foladlou et al. 2019). These agents can include chemical substances that inhibit nucleic acid and protein synthesis within the cell, disrupt mitochondrial energy production pathways, or compromise the integrity of cellular membranes, such as the plasma membrane or intracellular organelles with membranes (Li et al. 2019). Additionally, biological substances and physical agents can also exhibit cytotoxic effects, making it essential to consider a range of factors when evaluating the potential risks associated with exposure to toxic substances (Foladlou et al. 2019).

Cytotoxic agents can be classified into three categories: chemical, biological, and physical. Chemical cytotoxins include inhibitors of dihydrofolate reductase, DNA biosynthesis, and DNA strand break formation, as well as agents that induce DNA adducts and RNA degradation (Singh et al. 2018). Biological cytotoxins include toxins derived from viruses, bacteria, fungi, plants, and animals, such as lipid hydrolyzing enzymes, sphingomyelinases, and saponin (Al-Dabbas et al. 2015). Physical cytotoxins include heat, ultrasonic vibrations, and radiation, which can cause oxidative stress, mitochondrial membrane disruption, and synergistic enhancement of drug toxicity (Mishra et al. 2019). These agents can target various cellular components, including DNA, RNA, proteins, and the cell skeleton, leading to cytotoxic effects that can range from growth inhibition to cell death (Mishra et al, 2019).

Cell death can occur through various mechanisms, including apoptosis, autophagy, and necrosis (Ahmed et al. 2014). Apoptosis, or programmed cell death, is a regulated process that removes damaged or excess cells, ensuring tissue homeostasis (Li et al. 2019). Autophagy, or self-devouring, is a natural process that extracts and degrades cellular components, allowing for the recycling of cellular materials. Necrosis, on the other hand, is a form of cell injury that

results in premature cell death due to factors such as infection, toxins, or trauma (Kumar et al. 2020). Necrotic cell death is not a controlled process and can lead to inflammation and tissue damage. Necroptosis is a recently discovered programmed necrosis pathway that is distinct from apoptosis. While apoptosis acts as a cellular quality control mechanism, autophagy serves as an intracellular quality control mechanism (Kumar et al. 2020). The three pathways are not interchangeable and can occur simultaneously, with autophagy playing a role in both survival and cell death. Understanding the mechanisms of cell death is crucial for understanding disease development and treatment strategies (Li et al. 2015).

Cytotoxicity testing is a critical method for biological evaluation, offering several advantages, including its preferred and mandatory nature. The evolution of cytotoxicity testing has introduced various methods, including morphological assessments of cell damage, quantification of cell damage, and measurement of cell growth and metabolic properties, shifting from qualitative to quantitative evaluation (Erman et al. 2019).

Cytotoxicity tests measure the reduction in cell viability by inhibiting growth or inducing cell death through apoptotic-necrotic pathways. These assays can be performed *in vitro* and/or *in vivo* using various techniques. Some parameters, such as mitotic index, replication index, and nuclear division index, indirectly assess cell division dynamics, while others, like MTT, MTS, XTT, and WST, directly measure cell viability (Shaibani et al. 2019)

One of the most widely used cytotoxicity tests is the MTT/XTT assay, which assesses cell viability by measuring cellular metabolic activity (Liu et al. 2018), and the *Allium cepa* test which evaluate disturbances of the mitotic cycle and root growth inhibition (Liman et al. 2011).

8. Genotoxicity

Genotoxicity is a type of toxicological manifestation that arises from damage to genetic material, including DNA and RNA, caused by exposure to internal or external agents (Lee et al. 2017). This damage can manifest as small lesions forming at specific sites within DNA strands, resulting in DNA adducts, cross-links, and mutations. Alternatively, gross chromosomal abnormalities can occur, including changes in chromosome copy number, fragmentation, and structural rearrangements (Yang et al. 2021). The nature of genotoxic effects depends entirely on the mechanism of action of the agent responsible. (Ahmed et al. 2014). Genotoxicity refers to the ability of xenobiotics to induce genetic damage by interacting with the DNA molecule, thereby compromising its structure and function (Armel et al. 2014).

Genotoxic agents can be categorized into three main types based on their origin: physical, chemical, and biological (Lee et al. 2017) Physical genotoxins are substances that can cause DNA damage through physical interactions, such as radiation, UV light, or high-temperature

exposure. Chemical genotoxins, on the other hand, are substances that can react with DNA molecules to alter their structure or function, such as alkylating agents, electrophiles, and oxidative stressors. Biological genotoxins are living organisms or products of living organisms that can cause genetic damage, including viruses, bacteria, and other microorganisms (López-Romero et al. 2018).

These types of DNA damage can have serious consequences, including genetic mutations or cell death (Hastings et al. 2019). However, there are mechanisms in place to repair this damage. For example, nucleotide excision repair and base excision repair are two types of repair systems that can fix damaged DNA (Chatterjee et al. 2017).

Genotoxicity testing is an essential toxicological endpoint used to evaluate the potential of harmful substances to damage genetic material. This type of testing is crucial for assessing the biosafety of substances, both in living organisms *in vivo* and in laboratory experiments *in vitro* (Radhika et al. 2019).

The Ames assay, which employs histidine-auxotrophic mutants of Salmonella typhimurium, is one of the most widely used genotoxicity tests (Chatterjee et al. 2017). Additionally, the Comet assay is a microelectrophoresis technique that detects single and double-strand breaks in DNA by lysing cells and suspending them in an agarose gel. Two other common genotoxicity tests are the chromosomal aberration assay and the micronucleus assay, which assess chromosomal damage and disruption in the mitotic apparatus during cell division stages, respectively (López-Romero et al. 2018).

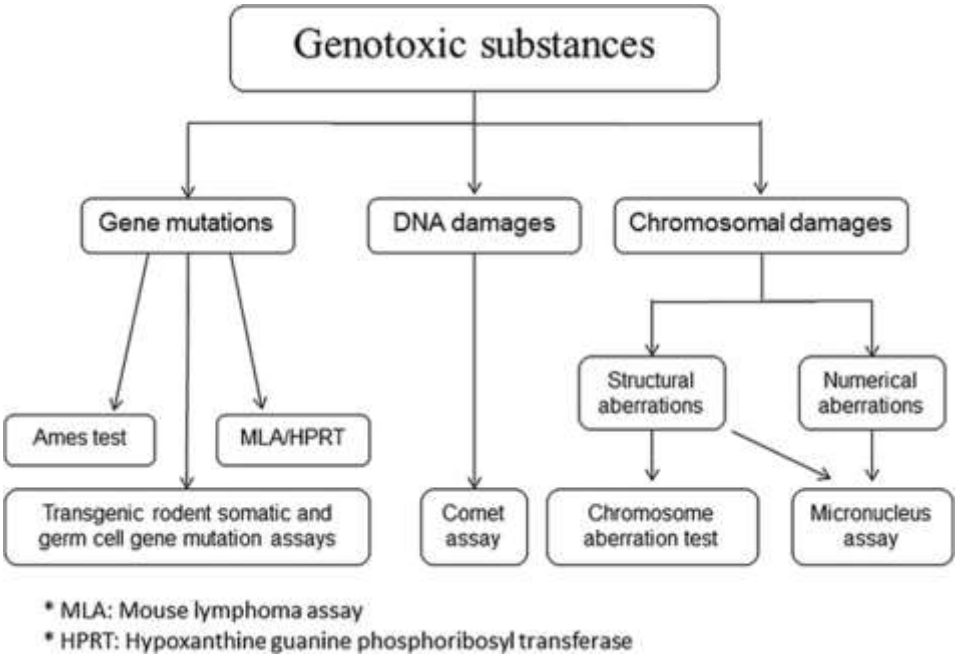

Figure 4. The most common testing methods for the assessment of genotoxic substances.

The assessment of genotoxicity and mutagenicity plays a crucial role in the evaluation of the potential hazards posed by various substances, including therapeutic drugs, cosmetics, agrochemicals, industrial compounds, food additives, natural toxins, and nanomaterials. These tests are essential for regulatory purposes, enabling authorities to identify and mitigate potential risks to human health and the environment (Hasan et al. 2017).

9. *Allium cepa* assay

Onion (*Allium cepa*) is a commonly used *in vitro* model to evaluate the cytotoxicity and genotoxicity of chemical substances (Kaur et al. 2018). This is achieved by allowing developing onion roots to come into contact with the substances being tested, thereby allowing for an assessment of their potential harmful effects on the cells and DNA (Kusumaningrum et al. 2012).

The *Allium cepa* test utilizes the root meristem cells of onion as a sensitive indicator of genetic damage caused by various chemicals or environmental pollutants. By measuring chromosome aberrations (CAs) in these cells, researchers can detect breaks, gaps, multiple breaks, chromatid breaks, ring chromosomes, dicentric chromosomes, and changes in chromosome number (Kaur et al, 2018). These abnormalities serve as a proxy for assessing the genotoxic potential of substances under investigation (Firbas and Tomaž 2014).

The *Allium cepa* test has been widely regarded as a low-cost and easy-to-handle assay, offering several advantages over other short-term tests used in environmental monitoring. Its ability to expose organisms directly to complex mixtures without prior treatment makes it a valuable tool. The test's oxidase enzyme system plays a crucial role in evaluating promutagens, which require metabolic activation to become mutagenic (Souza et al. 2018) The *Allium cepa* plant's metabolic capacity allows it to activate promutagens into mutagens, making it possible to assess this contaminant class without the need for an external metabolic system. This test is capable of detecting environmental genotoxic and mutagen quickly and sensitively, offering insights into genetic endpoints and mechanisms of action on DNA (Leme and Marin-Morales 2009).

The common onion has emerged as the most popular choice for the test, repeatedly recommended as a standard test material due to its reliability and versatility (Kaur et al. 2018).

Methodology

1. Preparation of plant extracts

Needles of *Juniperus oxycedrus* L., collected from the forest of Faïdja region (Tiaret, Algeria), were cleaned of any impurities then air dried in the dark at room temperature. After that, needles were crushed in an electric blinder to generate a somewhat soft powder that was stored in a well-covered jar and kept out of light.

Figure 5. Steps of material plant preparation.

2. Preparation of plant extracts

2.1. Aqueous maceration

The aqueous extract was prepared through macerating 50 g of plant powder and 500 mL of distilled water in a glass flask. The mixture was left with stirring at room temperature in the dark for 24 hours. The extract was then filtered using Whatman paper, and the obtained filtrate was dehydrated in an incubator at 37 °C to obtain a dry residue, which was then stored in a clean box.

Figure 6. Aqueous extract.

2.2. Maceration with ethanol

The ethanolic extract was prepared by putting 33.5 g of powder in a glass flask containing 335 mL ethanol 70 %. The mixture was agitated for 3 hours and then macerated for 24 hours. The resulting extract was filtered using Whatman paper, and the filtrate was dehydrated in an incubator at 37 °C to obtain a dry residue, which was then stored in a clean container.

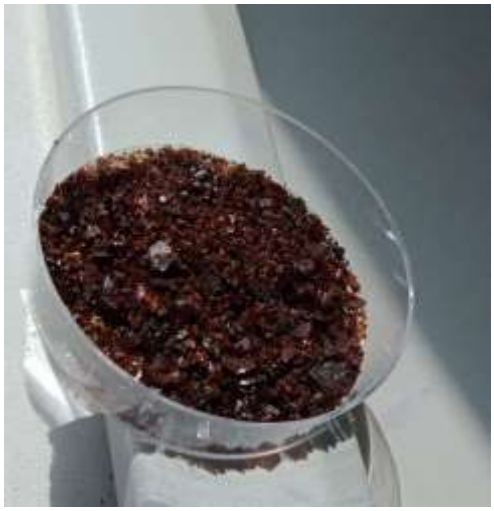

Figure 1. Ethanolic extract.

Extraction yield is the proportion of the amount of extracted analyte to the quantity of plant sample:

Yield (%) = Weight of the dry extract x 100/ Initial weight of the dry plant.

2. Phytochemical compounds

2.1. Polyphenols

Using a micropipette, a volume of 200 µL of each extract (aqueous and ethanolic) with the same concentrations as before (0.1, 0.5, 0.75, 1 mg/mL), was added to Eppendorf tubes along with a ten-fold diluted 1 ml of Folin-Ciocalteu reagent. The tubes are shielded from light and incubated for 5 min at room temperature. Next, each tube is filled with 800 µl of a 7.5% sodium carbonate (Na2CO3) solution. After shaking, the tubes are held for 30 min. The absorbance is measured at 765 nm. The total polyphenol content is derived from the calibration curve, and the findings are expressed as mg gallic acid equivalent (GAE)/mL of the extract. Each experiment was replicated a minimum of three times.

2.2. Flavonoids

In an Eppendorf tube, 1 mL of each extract was mixed with 1 ml of Aluminum chloride ($AlCl_3$) with 2% methanol. The solution is forcefully stirred using a vortex before being incubated in the dark for 15 minutes. The absorbance is instantaneously measured at 430 nm against a blank (distilled water). The total flavonoid content is estimated by utilizing a calibration curve, and the findings are presented as mg quercetin equivalent (QE)/mL extract. Each experiment was repeated a minimum of three times.

2.3. Tannins

Each extract was added in a volume of 50 µL to 1500 µL of the 49% vanillin/methanol solution and then mixed with force. Afterward, 750 µl of concentrated hydrochloric acid (HCl) was added. For 20 min, the resulting mixture is set to react at room temperature. Absorbance is measured at 550 nm to a blank. The overall tannin content is assessed using a calibration curve, and the results are indicated as mg quercetin equivalent (TAE/mL) extract. Each experiment was replicated at least three times.

3. Antioxidant activity using DPPH radicals scavenging assay

The radical scavenging activity of the extract was assessed utilizing 1,1-diphenyl-2-picrylhydrazine (DPPH). 0,2 ml of plant extract was put in Eppendorf tubes, then 1 ml of DPPH solution (2mg in 100 ml of methanol) was added. The tubes were incubated in the dark for 30 minutes. After the incubation, the absorbance measurements for each sample were set to 517 nm.

DPPH Scavenged (%) = [(Acontrol – Asample)/ Acontrol] × 100

4. Mitotic index analysis

Onion plant, *Allium cepa* (2n = 16), is a member of the Alliaceae family. Onion bulbs were purchased from a local market in Tiaret (Algeria) according to their size and were cleaned in the distilled water, after removing the roots and first peel. Prior to initiating the *Allium* anaphase-telophase test, outer scales of the bulbs and the dry bottom plate were removed without destroying the root primordia. After 2 days of contact with the distilled water in the darkness at 22 ± 4 °C, bulbs which roots have a length from

1 to 2 cm were used for this study. This consists on placing each of the germinated bulbs on a test tube containing distilled water so that only roots are immersed.

After that, the best growing four bulbs, were exposed for 48 h to different aqueous and ethanolic concentrations of *Cedrus atlantica* solutions (0.1, 0.5, 0.75 and 1 mg/mL respectively). The concentrations used in this study are chosen to match those found in our previous ethnopharmacological studies regarding the traditional medicinal use of *Cedrus atlantica* in Algeria (Taïbi et al. 2021). Distilled water serves as negative control while methyl methane sulfonate (MMS, 10 ppm) was employed as positive control.

After 48 h of exposure in the darkness, the last 2 cm of root was taken and fixed in Carnoy's solution at 4 °C for at least one night and then stored for long term in ethanol 70 % at 4 °C. These roots were then hydrolyzed in a solution 1 N HCl during 8 min and stained with Feulgen dye shielded from the light (Silva et al. 2011).

Results

1. Evaluation of the yield of extraction

The table 1 shows that the amount of extract obtained from the plant is greater for the ethanolic extract compared to the aqueous extract.

Table 1. Yield of the plant extracts.

Extract	Yield %
Aqueous extract	9.3
Ethanolic extract	12,86

2. Phytochemical compounds

2.1. Total phenolic content

The examination of the data reveals a notable difference in the total phenolic content among aqueous and ethanolic extracts in the various studied concentrations (Fig. 11).

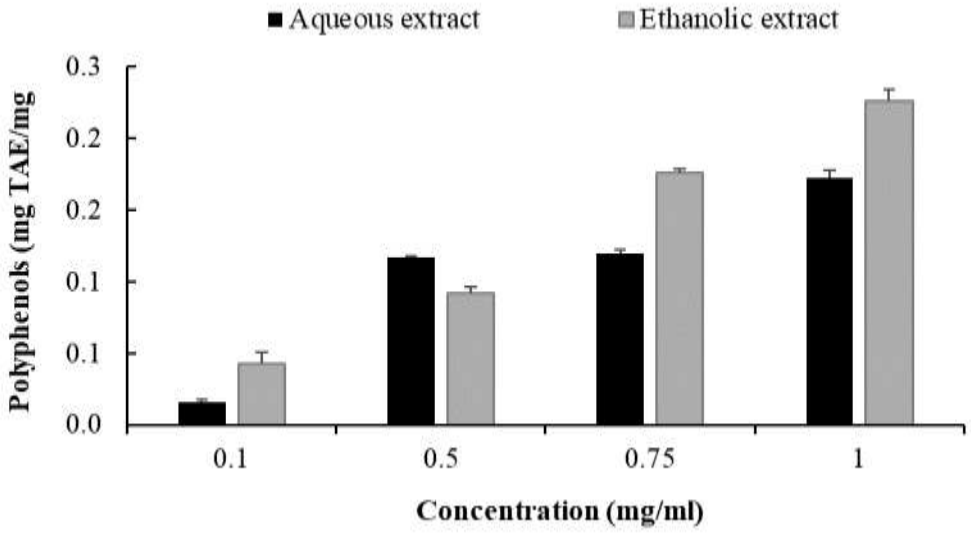

Figure 1. Variation of total phenolic contents in the aqueous and ethanolic extracts of *J. oxycedrus* L.

In general, the ethanolic extract has a higher content of phenolic content than the aqueous extract. The higher content of phenolic compounds is observed in aqueous extract (0,17± 1 mg QE/g) in the treatment with 1 mg/mL. However, the lowest content (0,02 ± 0,1 mg QE/g) is marked

in the treatment with 0.1 mg/mL. The phenolic content ranged between 0,23± 0,75 mg QE/g and 0,04± 0,1 mg QE/g respectively in the treatments with 1 and 0.1 mg/mL of ethanolic extract.

2.2. Flavonoids content

The study found that the plant extracts have different amounts of flavonoid compounds. he ethanolic extract has a higher content than the aqueous extract.

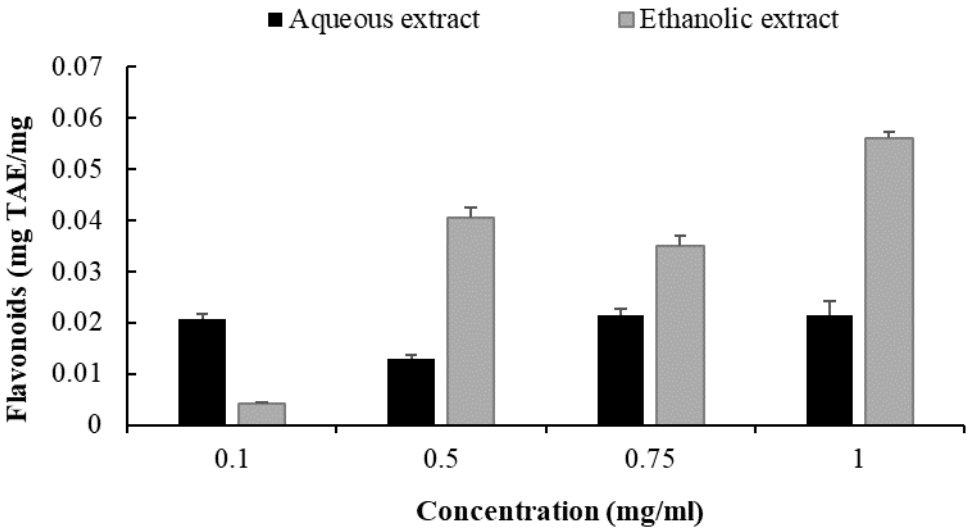

Figure 2. Flavonoids content of the tested plant extracts.

The lower content of flavonoids in the aqueous extract 0,013±0,004 mg QE/g was observed in the treatment 0.5 mg/mL. However, the highest value 0,02±0,007 mg QE/g was marked in the other treatments. Regarding the ethanolic extract, the highest content of flavonoids 0,06± 0,75 mg QE/g was observed in the treatment 1 mg/mL, while the lowest value was observed in the treatment 0,1 mg/mL.

2.3. Tannins

The examination of the data reveals a notable variation in the tannin content among the plant extracts being studied.

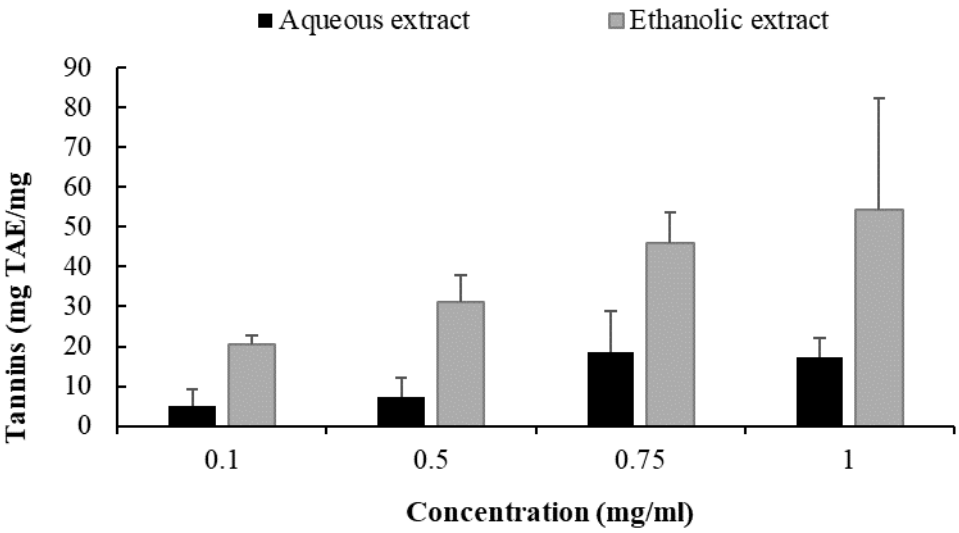

Figure 3. Tannins content for the tested plant extracts.

In general, for both extracts, the content of tannins increased when the concentration of plant extract increase. In addition, the ethanolic extract has a higher content than the aqueous extract. It ranged between 4,97 ± 0,1 mg QE/g and 18,62± 0,75 mg QE/g respectively in the lowest and highest concentration of the aqueous extract. However, it ranged between 20,37± 0,1 mg QE/g and 54,33±1 mg QE/g in the same treatments of the ethanolic extract.

3. Antioxidant activity

The antioxidant activity was measured by the DPPH scavenging assay. In general, the antioxidant activity was higher in the ethanolic extract in comparison with the aqueous extract (Fig. 15).

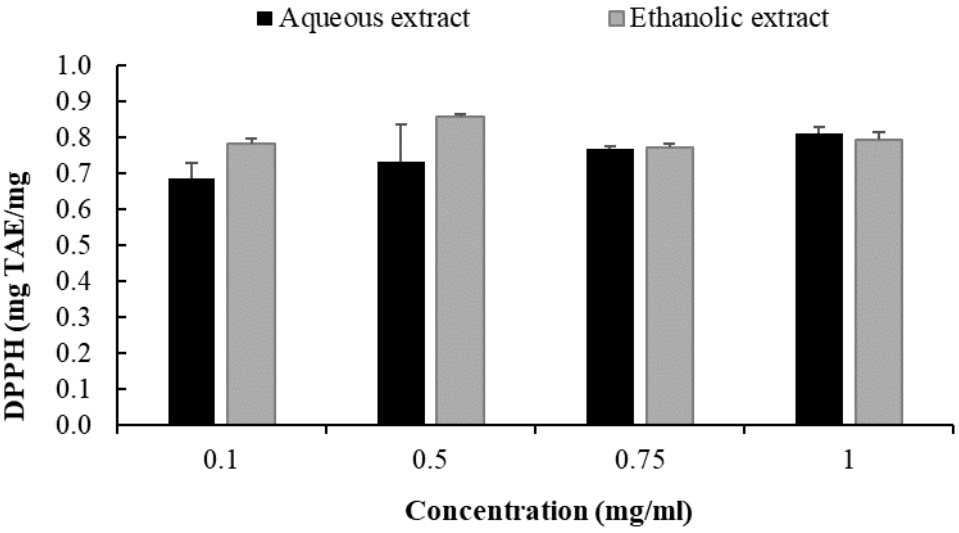

Figure 4. Variation of antioxidant activity (DPPH) for the tested plant extracts of *Juniperus oxycedrus* L.

The highest antioxidant activity was observed in the treatment of 0.5 mg/mL for ethanolic extract and in the treatment of 1 mg/mL for the aqueous extract. The scavenging activity ranged between 66.54% and 83.99% for the aqueous extract and between 78.38 and 86.4 % in the ethanolic extract.

4. Evaluation of the cytotoxicity

4.1. Root morphometry

- Roots number

The number of roots of *Allium cepa* was counted at two points in time: before and after adding plants extract to the growth medium for each treatment (Fig.15).

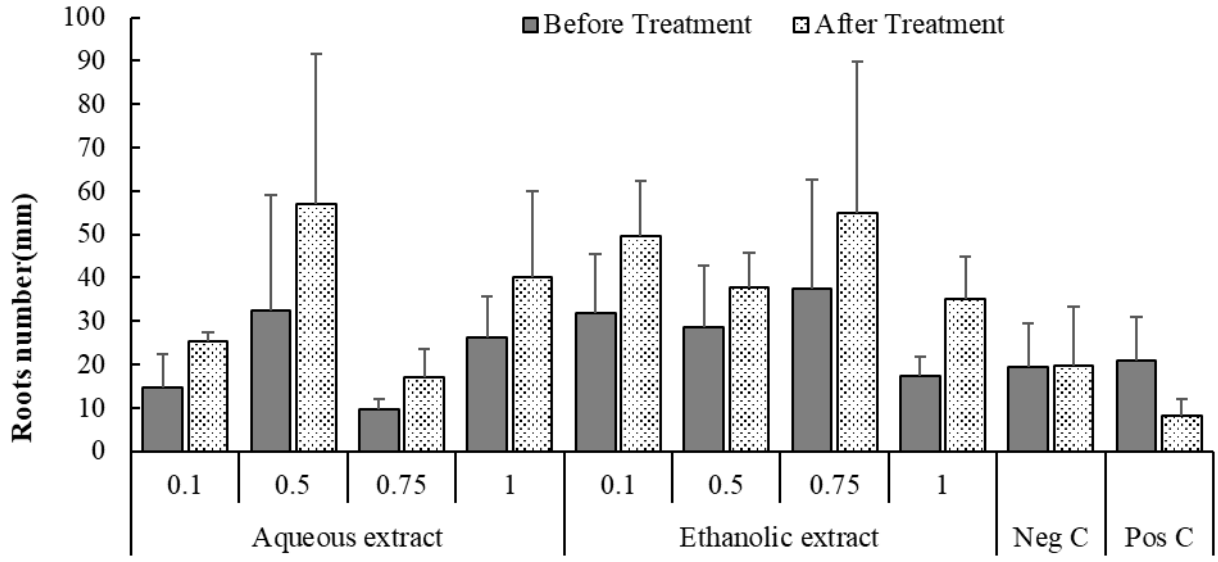

Figure 5. Variation of root number of *Allium cepa* under the effect of *Juniperus oxycedrus* L. extracts.

Root numbers increase in both extracts only in the treatments with ethanolic extract at concentrations below 0.75 mg/mL but no tendency was observed in the treatments with aqueous extract. The number of roots remained constant (20 roots) in the negative control group treated with distilled water. However, it varies according to the concentration of the extract in the medium.

- Root length

Root length was measured before and after adding plant extracts (fig.16). Root growth stopped with MMS solution, but tripled in length when using distilled water (the control).

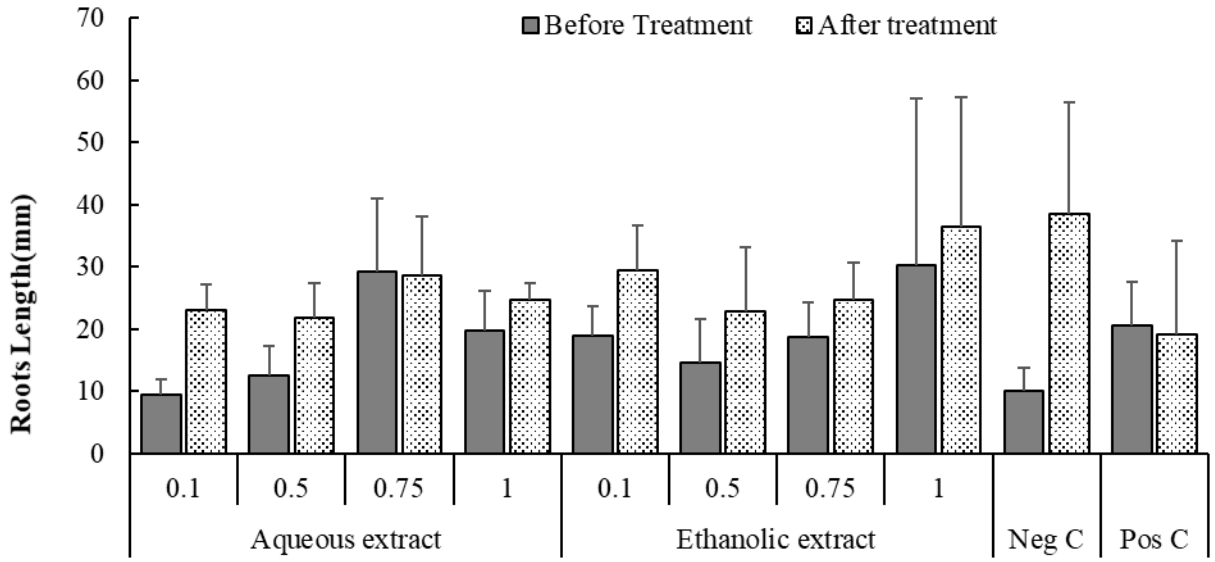

Figure 6. Variation of root length of *Allium cepa* under the effect of *Juniperus oxycedrus* L. extracts.

The number of roots increases in the negative control however, it remains unchanged after the treatment with MMS solution. The aqueous extracts showed increases in root length till the concentration 0.75 mg/mL. however, a systematic increase was observed in the ethanolic extract.

- Root diameters

Root diameter decreased in both positive and negative control groups (Fig. 17). Furthermore, the root diameter of plants treated with ethanolic extract showed a significant increase. The maximum root diameter was observed in the treatment with 0.75 mg/mL ethanolic extract, measuring 1.2 mm.

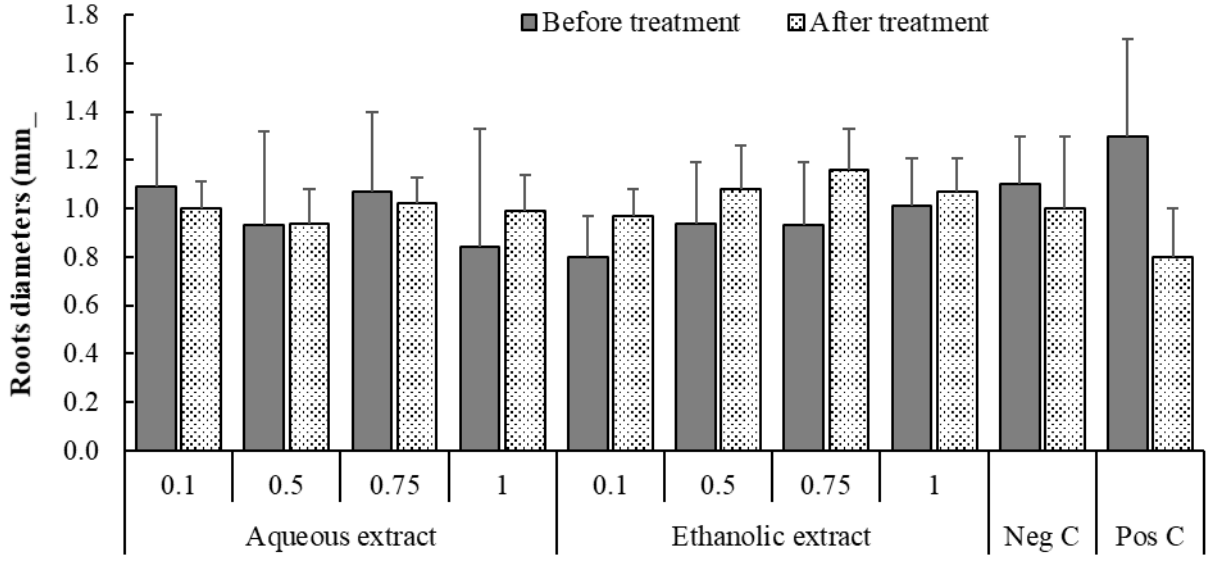

Figure 7. Variation of root diameter of *Allium cepa* under the effect of *Juniperus oxycedrus* L. extracts.

The aqueous extracts didn't change much in terms of root diameter. The ethanolic increased root diameter across all the tested concentrations.

4.2. Cell phases

- Mitotic index

The MI was higher in the treatments with plant extracts in comparison to the negative control with distilled water (Fig. 19).

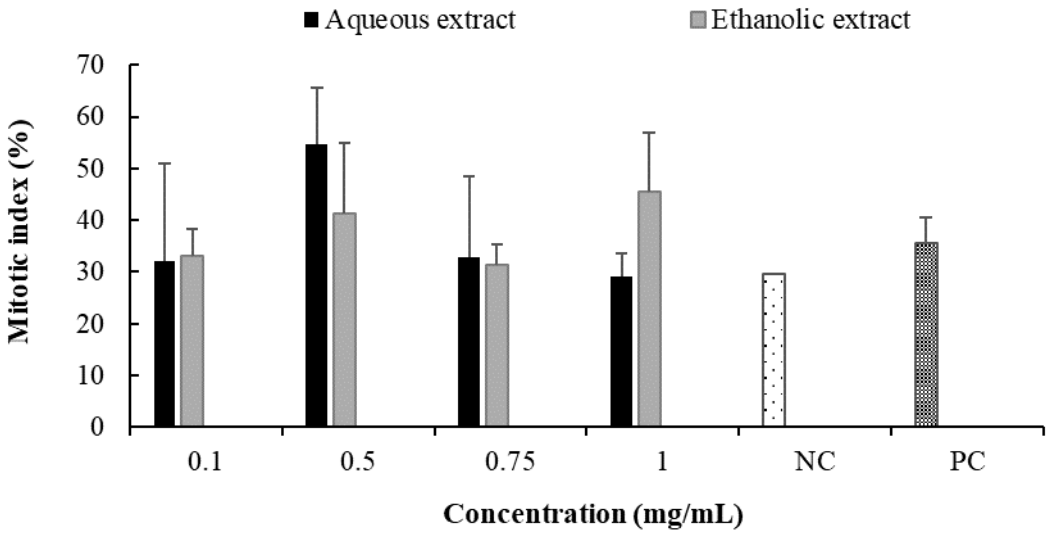

Figure 8. The effect of *Juniperus oxycedrus* extracts on mitotic index (MI) and mitotic phases in the root cells of *A. cepa*.

The ethanolic extract generally shows higher values of MI at all concentrations. The highest value of the MI is observed in the treatment with 1 mg/mL of the ethanolic extract and in the treatment with 0.5 mg/mL of the aqueous extract

- Prophase

The number of cells in prophase increases after the addition of both extracts comparing to the negative control. Comparing the numbers of prophase between the aqueous and ethanolic extracts, the aqueous extract exhibits higher counts at concentrations of 0.1, 0.5, and 0.75 mg/mL. However, at 1 mg/mL, the ethanolic extract shows a higher count (Fig. 20).

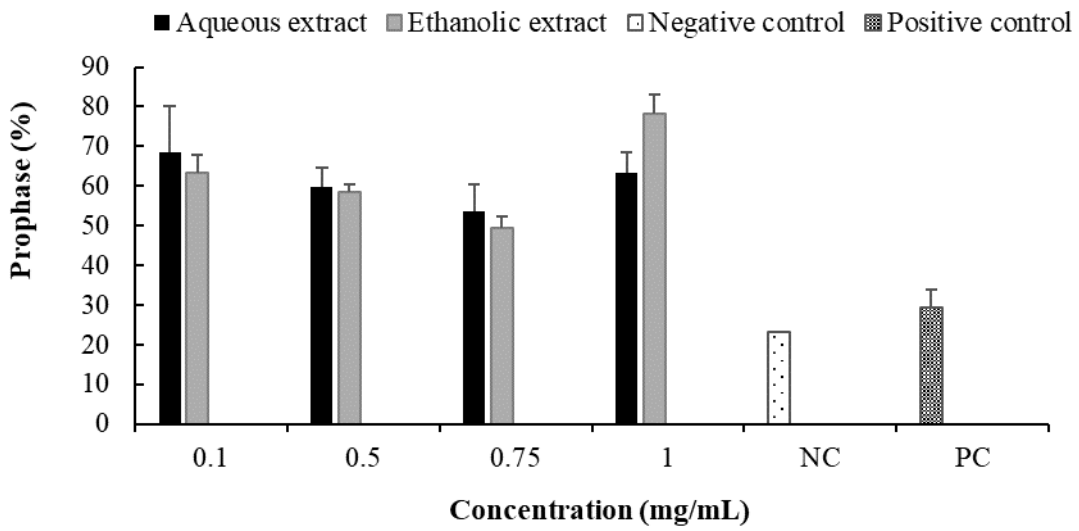

Figure 9. The effect of *Juniperus oxycedrus* extracts on prophase in the root cells of *A. cepa.*

This suggests that the aqueous extract is more effective at lower concentrations, while the ethanolic extract demonstrates greater effectiveness at the highest concentration tested.

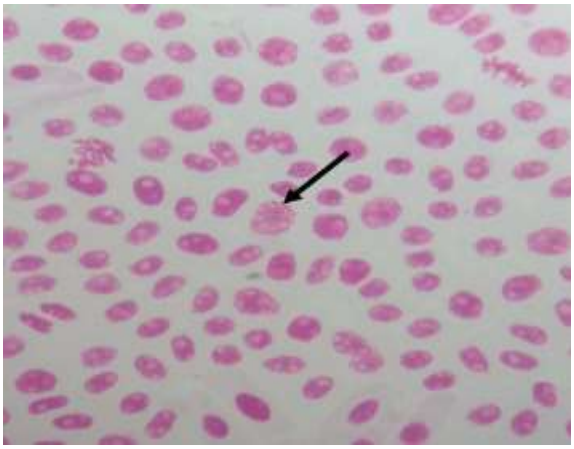

Figure 10. Normal prophase (© 2024).

- Metaphase

The number of cells in metaphase increases in the treatments of both extracts (Fig. 22). It reaches the maximum under the treatment with 0.5 mg/mL for both extracts then drop below this concentration.

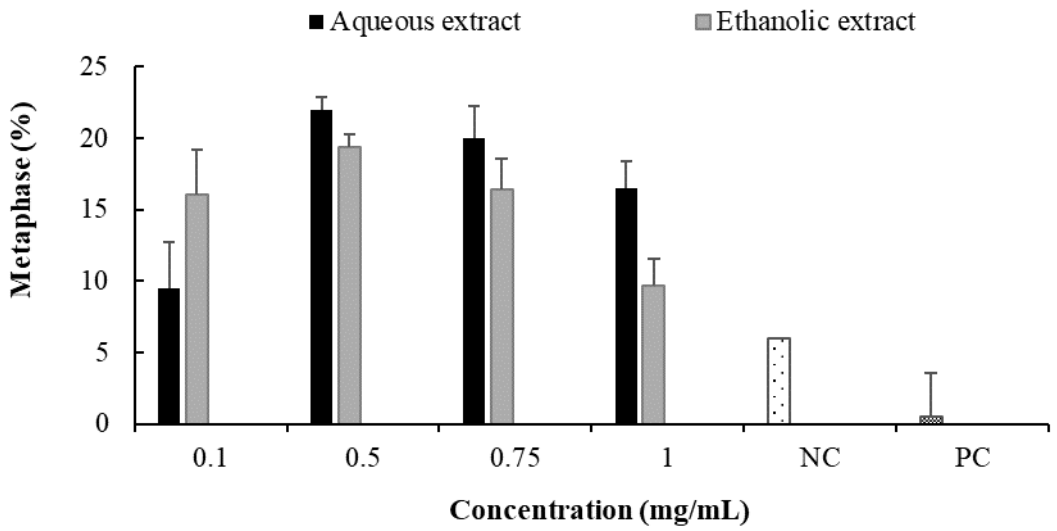

Figure 11. The effect of *Juniperus oxycedrus* extracts on metaphase in the root cells of *A. cepa*.

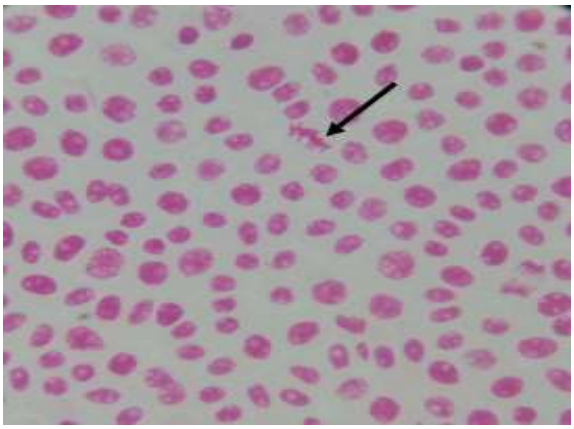

Figure 12. Normal metaphase (© 2024).

- Anaphase

The number of cells in anaphase increases significantly in treatments with both aqueous and ethanolic extracts. The highest cells number was observed under treatment with 0.5 mg/mL.

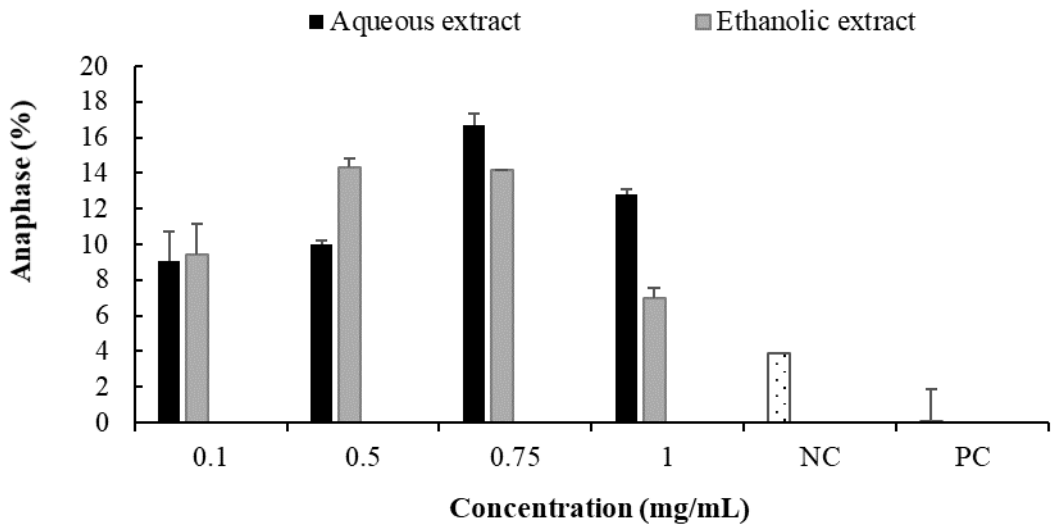

Figure 13. The effect of *Juniperus oxycedrus* extracts on anaphase in the root cells of *A. cepa.*

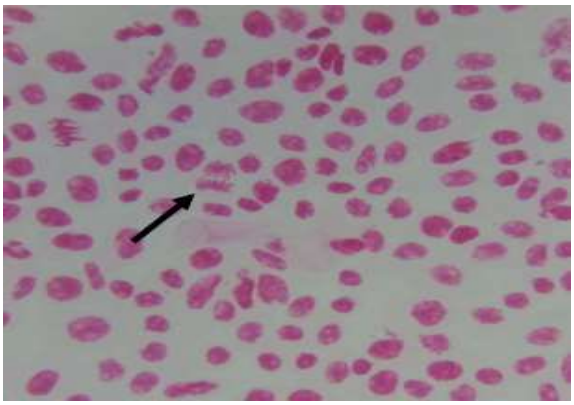

Figure 14.Normal anaphase (© 2024).

- Telophase

The number of cells in telophase was higher in treatments with both extracts comparing to control (Fig. 26). It increases systematically in the aqueous extract according to the concentration of the extract to range between a minimum of 1.2% and a maximum of 2.5%. However, the increased tendency observed under treatments with ethanolic extract was marked by a higher value observed in treatment with 0.5 mg/mL.

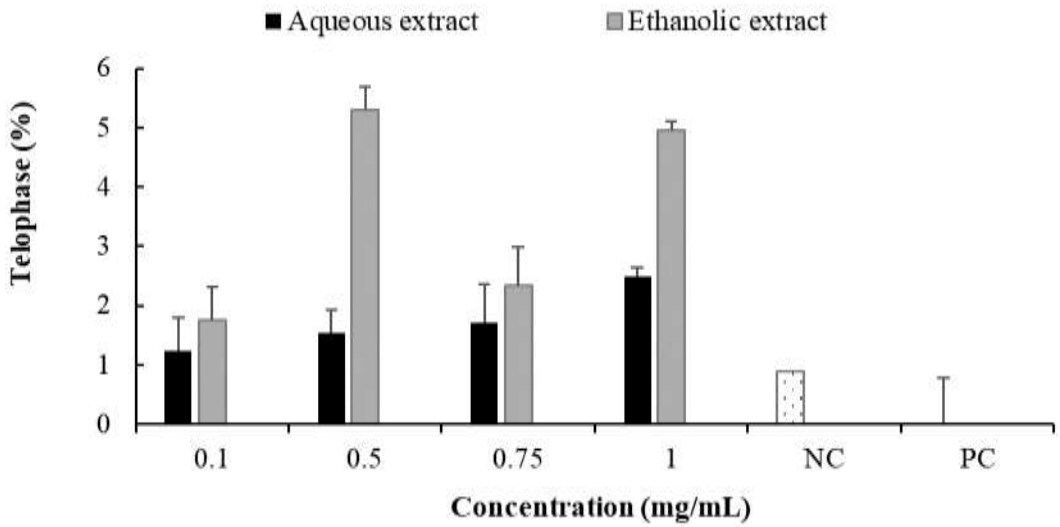

Figure 15. The effect of *Juniperus oxycedrus* extracts on telophase in the root cells of *A. cepa.*

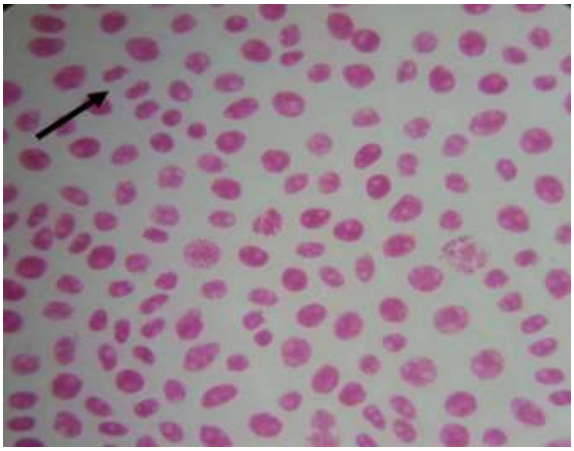

Figure 16. Normal telophase (© 2024).

Microscopic examination of *Allium cepa* root tips revealed chromosomal abnormalities after treatment with *Juniperus oxycedrus* extracts. Our analysis showed that both the water and ethanolic extracts had significant effects on the chromosomes of *A. cepa* root tips, with the ethanolic extract inducing more aberrations (427) than the aqueous extract (203).

Telophase adherence and binuclear cells, both of them is present in either extract. The frequency of (C-Metaphase, Polyploidy, Micronucleus, Metaphase Adherence) was higher compared to the overall rate of Anaphase - Telophase anomalies (Disturbed Anaphase - chromosome laggard -chromosomic bridge) with decreasing doses, the frequency of chromosomal anomalies increases, resulting in a lower incidence of anomalies at higher concentration.

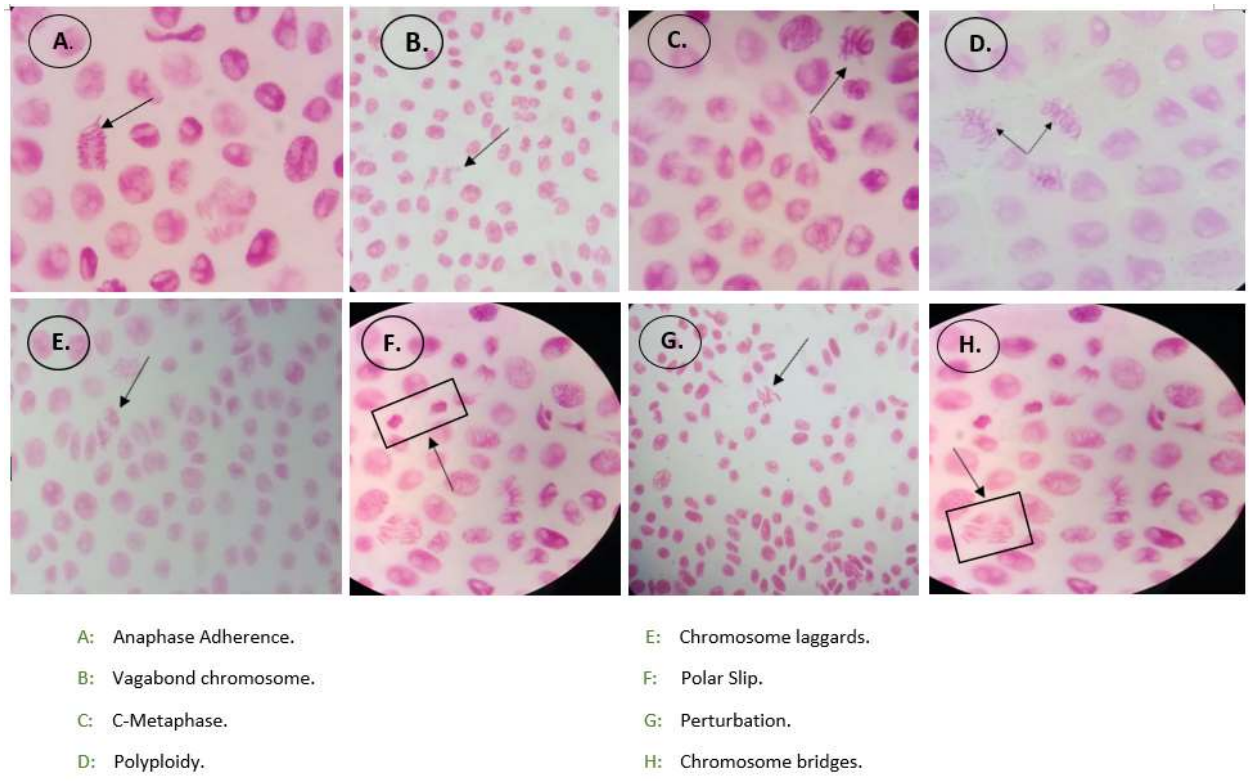

Figure 17. Chromosomal aberrations observed in *Allium cepa* treated with *J. oxycedrus* extracts (© 2024).

Micronucleus has the lowest rate with a percentage of 1.88%, while the most chromosomal anomalies was observed in polyploidy with a percentage of 31.75% accompanied by chromosomic bridges, disturbed anaphase, with 12,75%,10.13% respectively, then we have C-Metaphase, chromosome laggard in the same rank with a percentage of 7.50%, metaphase adherence had a 6 % of chromosomal anomalies in this test. The highest number of chromosomic bridges was observed in samples treated with 1 mg/mL of the aqueous extract with 17 anomalies detected. In contrast, the highest frequency of abnormalities in the ethanolic extract was at 0.5 mg /mL with 32 anomalies.

Discussion

Discussion

For centuries, medicinal plants have been an integral part of healthcare, with a substantial portion of the global population relying on botanical preparations to meet their health needs (Ghorbani 2017). As people become more interested in natural products, scientists are now looking at how plant compounds can help make people feel better (Pandey et al. 2009).

Juniperus oxycedrus has been widely utilized in traditional folk medicine to treat various infectious diseases, chronic eczema, and other skin conditions, as well as hyperglycemia, obesity, tuberculosis, bronchitis, and pneumonia; cade oil, derived from this plant, is also used as a fragrance component in soaps, detergents, creams, lotions, and perfumes (Al-Snafi and Esmail 2018). *Juniperus oxycedrus* berries have been traditionally employed for the treatment of gastrointestinal disorders, respiratory issues, calcinosis, urolithiasis, urinary tract infection and hemorrhoids (Akkol et al. 2009).

Ethnobotanical studies in Turkey show that decoction of *Juniperus oxycedrus* used to treat diabetes and hyperglycemia (Orhan et al, 2012). The use of medicinal plants may pose potential health risks (Godwin et al. 2022). However, Taïbi et al. (2020, 2021) reported its traditional uses to manage skin disorders, urinary diseases and cancers in Algeria.

The primary goal of this investigation is to examine the cytotoxic and genotoxic potential of *Juniperus oxycedrus* using the *Allium cepa* test. Additionally, the study aims to assess the antioxidant properties of the plant and quantify its major secondary metabolites, including polyphenols, flavonoids, and tannins.

The highest yield of extraction was achieved from the ethanolic extract. It is challenging to directly compare the findings of this study with those in the literature due to the variability in yield, which is influenced by factors such as the chemical composition of the plants, extraction processes (including temperature, time, and pH), and the polarity of the solvents employed (Toul et al. 2022).

The results indicate a significant difference in polyphenol and flavonoids contents among the tested extracts. Similar findings were reported by Reda et al. (2019). The tannins content of Juniper leaves has been found to vary depending on the location of the plant (Smeriglio et al. 2019). Lachowicz et al. (2008) explored the effectiveness of two methods for extracting tannins from Juniper leaves: infusion and maceration. The researchers found that both methods were capable of extracting tannins, but the maceration method yielded higher levels of tannins than the infusion method.

The DPPH assay is a widely used and preferred method for evaluating the antioxidant activity of various compounds and extracts due to its ease of use, high sensitivity, excellent reproducibility, and simplicity. The obtained results revealed that the evaluated extracts exhibit significant antioxidant activity present in the ethanolic extract more than the aqueous extract. El Jemli et al. (2016) found that the aqueous extract of *Juniperus oxycedrus* exhibited high antioxidant activity in DPPH, with IC_{50} values of 17.91±0.37 µg/mL and 0.07 µg/mL, respectively. The strong correlation between antioxidant capacity and total phenolic content suggests that phenolic compounds are a primary contributor to the antioxidant properties of these extracts.

The *Allium cepa* assay demonstrated genotoxic potential of the extracts assessed by examining the structural changes and numerical alterations of chromosomes in the meristem cells of young growing onion root tips, which serve as a model system for evaluating the mutagenic and carcinogenic effects of the agent under study (Firbas 2014).

The treatment of *Allium cepa* root tips with the plant extracts induced a spectrum of chromosomal aberrations, encompassing disturbances in anaphase-telophase progression, chromosomal lagging, fusion and bridging, as well as metaphase arrest, formation of binucleate cells, polyploidy, micronuclei, and other irregularities, suggesting genotoxic effects.

Among the chromosomal abnormalities observed, anaphase-telophase aberrations were found to occur at a lower frequency compared to other types of anomalies, including c-metaphase, binuclear cell formation, polyploidy, micronucleus formation, and metaphase adhesion. Maybe the higher concentrations of the extracts might induce cell cycle arrest predominantly at metaphase, thus preventing cells from progressing to anaphase and telophase. This would naturally result in fewer observed abnormalities in the later stages. The primary cause of c-metaphase is exposure to chemicals that disrupt microtubule formation (Barman, Manabendu 2020) leading to the arrest of chromosomes at metaphase and the inability of sister chromatids to separate during cell division.

Nishimura et al. (2016) showed that all 150 binucleated HeLa cells were formed by cytokinesis failure, with the two nuclei derived from a single mother cell. Live-cell imaging studies have shown that laggard chromosomes are caused by merotelic orientation, where a single kinetochore of the laggard chromosome has microtubule fibers connecting it to both spindle poles. This malorientation prevents the laggard from moving properly to one of the poles during anaphase (Janicke Marie et al. 2007). Our results showed that the ethanolic extract was more genotoxic than the aqueous extract at the higher concentrations (0,75 mg/mL. The results of an *in vitro* cytotoxic assay revealed a potent cytotoxic effect of the ethanolic extract against cancer cell lines, with a toxicity observed against normal cells.

A previous study demonstrated that *J. phoenicea* does not exhibit chromotoxic or mitodepressive effects on meristematic cells of *A. cepa* across the tested concentrations.

The mitotic index, a measure of the proportion of cells in the process of cell division, can be reduced due to cell death or delayed cell division. This decrease in mitotic index may be attributed to various factors, including chromatin dysfunction, blockage of the G2 phase, obstruction of prophase completion, arrest of one or more mitotic stages, or disruption of the cell cycle caused by inhibition of DNA synthesis and nucleoprotein dysregulation, which prevents cells from entering mitosis (El-Ghamery et al. 2000; Ihegboro et al. 2020).

Research has also shown that the inhibition of mitosis can be attributed to the inhibition of tubulin, which prevents the formation and progression of cell proliferation, thereby impeding the normal cell cycle process (Owolarafe et al. 2020).

Majewska et al. (2000) found that a decrease of the mitotic index may be caused by impaired nucleoprotein synthesis and a reduction in ATP levels, which are essential for providing energy for the processes of mitotic spindle elongation, chromosomal movement, and microtubule dynamics during cell division.

Conclusion

Conclusion

Medicinal plants remain a vital source of novels pharmaceuticals, serving as a rich repository of fundamental in gradients for the discovery of new compound that can lead to the development of innovative medication thereby contributing to the advancement of future treatment.

It is imperative that further research is conducted to fully understand the toxicological risks associated with *J. oxycedrus* as toxicity concerns have been observed. Until more is known, it is crucial to exercise caution and take necessary precautions when handling this plant species to ensure the safety of individuals involved in its cultivation, processing, and potential therapeutic applications. In contrast our research also focused on examining the phytochemical and biological aspects of *J. oxycedrus* extracts.

The present study demonstrates for the first time the cytotoxic and genotoxic effects of *J. oxycedrus* aqueous and ethanolic extracts using *Allium cepa* test. A significant genotoxic effect was observed in the ethanolic extract compared to the aqueous extract at concentrations above of 0.5 mg/mL.

Precautions must be taken due to toxicity concerns. Certainly, more studies are required to investigate the toxicological potential of this plant species.

References

References

(n.d.).

A. M. Rodriguez-Mateos et al. (2018). Flavonoids and Cardiovascular Health. *Journal of Nutrition*, 2243-2253.

A. M. Rodriguez-Mateos et al.,. (2018). Flavonoids and Cardiovascular Health". *Journal of Nutrition*, Journal of Nutrition.

Ahmed et al. (2018). Antimicrobial activity of Juniperus oxycedrus aqueous extract against microorganisms. *Journal of Pharmacy and Pharmacology*, 1325-1334.

Ahmed et al. (2018). Cytotoxic effects of Juniperus oxycedrus ethanolic extract on human cancer cell lines. *Journal of Pharmacy and Pharmacology*, 1325-1334.

Ahmed Nabile, et al. (2014). Toxicity of Plasmonic Nanomaterials and Their Hybrid Nanocomposites. *Advances in Molecular Toxicology*, 173- 202.

akkol et al. (2009). A comparative study on the antinociceptive and anti-inflammatory activities of five Juniperus taxa. *J.Ethnopharmacol.*, 330–336.

Ali et al. (2019). Induction of apoptosis by Juniperus oxycedrus ethanolic extract in human cancer cells. *ournal of Natural Products*, 533-542.

Ali et al. (2023). Anti-inflammatory activity of polyphenolic extracts of Juniperus oxycedrus. *Ethnopharmacology*, 281.

Al-Snafi and Ali Esmail. (2018). PHARMACOLOGICAL AND THERAPEUTIC EFFECTS OF JUNIPERUS OXYCEDRUS- A REVIEW. *INDO AMERICAN JOURNAL OF PHARMACEUTICAL SCIENCES*, 2198- 205.

Al-Snafi, Ali Esmail. (2018). PHARMACOLOGICAL AND THERAPEUTIC EFFECTS OF JUNIPERUS OXYCEDRUS-A REVIEW. *INDO AMERICAN JOURNAL OF PHARMACEUTICAL SCIENCES*, 2198 - 205. doi:doi.org/10.5281/zenodo.1214996.

APG. (2016). APG IV. *Botanical Journal of the Linnean Society*, 1-20.

APG/ Mark W. Chase, et al. (2016). APG IV. *Botanical Journal of the Linnean Society*, 1-20.

Armel Jackson, et al. (2014). Genotoxicity and Teratogenicity of African Medicinal Plants. *oxicological Survey of African Medicinal Plants, Elsevier*, 235- 75.

authors, n. (2013). *world health organization .* Retrieved from WHO: https://www.who.int/traditional-medicine/publications/2014-2023-traditional-medicine-strategy/en/

Barman, Manabendu. (2020). Colchicine Like Metaphase and Cell Cycle Delay Inducing Effects of Leaf Aqueous Extract of Clerodendrum Inerme (L.) Gaertn. in Allium Cepa Root Apical Meristem Cells. *CYTOLOGIA*, 197-201. doi:doi.org/10.1508/cytologia.85.197

Benhammou. (2009). Antioxidant activity of methanolic extracts and some bioactive compounds of Atriplex halimus. *Comptes Rendus Chimie*, 1259-1266. doi:doi.org/10.1016/j.exppara.2021.108155

Benzie et al. (2018). Chapter 1: Bioactive compounds in medicinal plants. In I. F.-G. Benzie, *Herbal medicine: Biomolecular and clinical aspects (3rd ed.)* (p. Chapter 1: Bioactive compounds in medicinal plants). Boca Raton, Florida, USA.: CRC Press.

Bouaziz et al. (2021). Bouaziz, S., Amri, M., Taibi, N., Zeghir-Bouteldja, R., Benkhaled, A., Mezioug, D., & TouilBoukoffa, C. (2021). Protoscolicidal activity of Atriplex halimus leaves extract against Echinococcus granulosus protoscoleces. Experimental Parasitology, 229, 1081. *Experimental Parasitology,*, 108-155. doi:doi.org/10.1016/j.exppara.2021.108155

Chatterjee et al. (2017). Mechanisms of DNA Damage, Repair, and Mutagenesis. *Environmental and Molecular Mutagenesis*, 235- 63.

chen et al. (2015). Phenolic acids from Juniperus oxycedrus: Isolation, identification, and evaluation of their antioxidant activity. *Food Science and Human Nutrition, 4(1), 33-40.*, 33-40.

Diversité des espèces de Juniperus L. (Cupressaceae) dans les régions montagneuses d'Algérie. (2010). *Revue d'Ecologie (Terre et Vie)*, 151-162.

El Jemli M et al. (2016). Radical-Scavenging Activity and Ferric Reducing Ability of Juniperus thurifera [L.], J. oxycedrus [L.], J. phoenicea [L.] and Tetraclinis articulata [L.]. *Adv Pharmacol Sci*, 6392-656. doi:doi: 10.1155/ 2016/ 6392656

El-Ghamery et al . (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*, 277-287.

El-Ghamery et al . (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*, 277-287.

El-Hawary et al. (2019). Antioxidant and free radical scavenging activities of Juniperus oxycedrus aqueous extract. *Journal of Food Science,*, 84(2).

Erman et al. (2019). Cell Division, Cytotoxicity, and the Assays Used in the Detection of Cytotoxicity. *Cytotoxicity - Definition, Identification, and Cytotoxic Compounds*. doi:https://doi.org/10.5772/intechopen.88368.

Firbas. (2014). Chromosome Damage Studies in the Onion Plant Allium Cepa L. *Caryologia*, 25-35. doi:doi.org/10.1080/00087114.2014.891696

Firbas, Peter, and Tomaž Amon. (2014). Chromosome Damage Studies in the Onion Plant Allium Cepa L. *Caryologia*, 25 -35. doi:https://doi.org/10.1080/00087114.2014.891696

Ghorbani, A. (2017). Review on Medicinal Plants Used in Traditional Medicine and Their Importance in Modern Medicine. *Journal of Pharmaceutical Research International*, 145-155. doi:10.9734/JPRI/JPRI-2017233301

Godwin Upoki et al. (2022). Cytotoxicity of Medicinal Plant Species Used by Traditional Healers in Treating People Suffering From HIV/AIDS in Uganda. *Frontiers in Toxicology*, 832-780. doi:doi.org/10.3389/ftox.2022.832780.

Hasan, et al. (2017). Genotoxicity Testing: Progress and Prospects for the next Decade. *Expert Opinion on Drug Metabolism & Toxicology*, 1089- 98. doi:https://doi.org/10.1080/17425255.2017.1375097

Ihegboro et al. (2020). Evaluation of the biosafety potentials of methanol extracts/fractions of Tapinanthus bangwensis and Moringa oleifera leaves using Allium cepa model. *Toxicology Reports*, 671-679. doi:doi: 10.1016/j.toxrep.2020.05.001

J. M. P. Q. Delić et al. (2018). Tannins: A Review of Their Biological Effects. *Journal of Food Science*, S1445-S1454.

Janicke Marie A et al. (2007). Chromosome Malorientations after Meiosis II Arrest Cause Nondisjunction. *Molecular Biology of the Cell, édité par Kerry Bloom*, 1645-56. doi:doi.org/10.1091/mbc.e06-10-0963

Kacem, K et al. (2010). Diversité des espèces de Juniperus L. (Cupressaceae) dans les régions montagneuses d'Algérie. *Revue d'Ecologie (Terre et Vie)*, 151-162.

Kacem, K., Boudiaf, A., & Bendiabdellah, F. (2010). Diversité des espèces de Juniperus L. (Cupressaceae) dans les régions montagneuses d'Algérie. *Revue d'Ecologie (Terre et Vie)*, 151-162.

Kumar et al. (2017). Traditional medicine: A review of the use of medicinal plants in traditional medicine. *Journal of Ethnopharmacology*, 1-12.

Kusumaningrum, Hermin Pancasakti, et al. (2012). Chromosomes and Mitotic Cell Division Phase In Onion Roots After 24 Hours Acetoorcein Soaking Time. *Bioma : Berkala Ilmiah Biologi,*, 46. doi:https://doi.org/10.14710/bioma.14.2.46-48

L. Vilar et al. (2016). *European Atlas of Forest Tree Species.* Retrieved from European Atlas of Forest Tree Species: https://w3id.org/mtv/FISE-Comm/v01/e013abb

Lachowicz et al. (2008). Tannin extraction from juniper leaves (Juniperus communis L.) using different solvents and methods. *Food Chemistry*, 666-672. doi:10.1016/j.foodchem.2007.09.044

Leme, D. M., and Marin-Morales, M. A. (2009). Allium cepa test in environmental monitoring: A review on its application. *Mutation Research/Reviews in Mutation Research*, 71-81. doi:https://doi.org/10.1016/j.mrrev.2009.06.002.

Li, Weijia, et al. (2015). Study of the in Vitro Cytotoxicity Testing of Medical Devices. *Biomedical Reports,*, 617- 20. doi:https://doi.org/10.3892/br.2015.481

Liu, Z., et al. (2018). Cytotoxicity testing: A review of current methods and their applications. *Journal of Pharmacy and Pharmacology*, 313-324.

López-Romero et al. (2018). Evidence of Some Natural Products with Antigenotoxic Effects. Part 2 Plants, Vegetables, and Natural Resin. *Nutrients*, 1954.

Majewska et al. (2000). Influence of extract from shoots of Taxus baccata var.'Elegantissima'on mitotic activity of meristematic cells of Allium cepa L. roots. *Acta Societatis Botanicorum Poloniae*, 185-192.

Nishimura et al. (2016). Binucleated HeLa Cells Are Formed by Cytokinesis Failure in Starvation and Keep the Potential of Proliferation. *Cytotechnology*, 1123-30. doi:doi.org/10.1007/s10616-015-9869-6.

Ogbera, A. O., & Agaba, E. I. (2010). Herbal medicine: a review of the current state of knowledge. *Journal of Medicinal Food*, 13(2).

Orhan et al. (2012). A Bioactivity Guided Study on the Antidiabetic Activity of Juniperus Oxycedrus Subsp. Oxycedrus L. Leaves. *Journal of Ethnopharmacology*, 409. doi:doi.org/10.1016/j.jep.2012.01.042

Pandey et el. (2009). Biotechnological perspectives of mushroom beta-glucans. *Food Technology and Biotechnology*, 141-154. doi:10.2478/v10050-008-0054-9

Patel et al. (2018). Anti-inflammatory and anti-pyretic activities of Juniperus oxycedrus aqueous extract. *Journal of Pharmacy and Pharmacology*, 63-72.

Patel et al. (2018). Biological activities of Juniperus oxycedrus. *ournal of Pharmacy and Pharmacology*, 63-72.

processOwolarafe et al. (2020). Investigation of cytotoxicity potential of different extracts of Ziziphus mauritiana (Lam) leaf Allium cepa model. *Toxicology Reports*, 816-821. doi:doi.org/10.1016/j.toxrep.2020.06.010

Radhika et al. (2019). A review on genotoxicity, its molecular mechanisms, regulatory testing in drug development process. *International Journal of Pharmaceutical Sciences and Research*, 4054-4069.

Reda et all. (2019). Phytochemical Characterization, Antioxidant and In Vitro Cytotoxic Activity Evaluation of Juniperus Oxycedrus Subsp. Oxycedrus Needles and Berries. *Molecules*, 502. doi:doi.org/10.3390/molecules24030502

Smeriglio et al. (2019). Chemical characterization of Juniperus communis L. leaves and berries: A comparative study. *Journal of Food Science*, S1465-S1473. doi:10.1111/1750-3841.14336

Toul et al. (2022). Algerian Bunium incrassatum Seeds: Effects of extraction solvent polarity on phenolic profile and antioxidant activity. *Journal of The Turkish Chemical Society Section A: Chemistry*, 475-482. doi:doi.org/10.18596/jotcsa.1058060

Toul, F., Djendar, A., Seladji, M., & Berroukeche, F. (2022). Algerian Bunium incrassatum Seeds: Effects of extraction solvent polarity on phenolic profile and antioxidant activity. *Journal of The Turkish Chemical Society Section A: Chemistry,*, 475-482. doi:doi.org/10.18596/jotcsa.1058060

Urquiaga and Leighton. (2000). Phenolic compounds in plants: an overview. *Phytochemistry*, 147-155.

WHO. (2013). *World Health Organization .* Retrieved from WHO: https://www.who.int/traditional-medicine/publications/2014-2023-traditional-medicine-strategy/en/

X. Wang et al. (2017). Flavonoids and Neuroprotection. *Journal of Agricultural and Food Chemistry*, 341-351.

X. Wang et al.,. (2017). Flavonoids and Neuroprotection. *Journal of Agricultural and Food Chemistry*, 341-351.

A. K. Singh et al. (2018). Cytotoxic agents: Mechanisms of action and therapeutic potential. *Journal of Pharmacy and Pharmacology*. doi:10.1111/jphp.12994

A. M. Rodriguez-Mateos et al. (2018). Flavonoids and Cardiovascular Health. *Journal of Nutrition*, 2243-2253.

Ahmed Nabile, et al. (2014). Toxicity of Plasmonic Nanomaterials and Their Hybrid Nanocomposites. *Advances in Molecular Toxicology*, 173- 202.

Ait-Ouhammou et al . (2018). Chemical composition and antimicrobial activity of Juniperus oxycedrus L. leaves. *Journal of Essential Oil Research 30(3)* , 235-244.

Al-Dabbas et al. (2015). Antimicrobial Activity of Juniperus oxycedrus Essential Oil Against Clinical Isolates. *Journal of Essential Oil Research*, 142-148. doi: 10.1080/10412905.2014.994343

Al-Snafi and Ali Esmail. (2018). PHARMACOLOGICAL AND THERAPEUTIC EFFECTS OF JUNIPERUS OXYCEDRUS- A REVIEW. *INDO AMERICAN JOURNAL OF PHARMACEUTICAL SCIENCES*, 2198- 205.

APG. (2016). APG IV. *Botanical Journal of the Linnean Society*, 1-20.

Armel Jackson, et al. (2014). Genotoxicity and Teratogenicity of African Medicinal Plants. *oxicological Survey of African Medicinal Plants, Elsevier*, 235- 75.

Chatterjee et al. (2017). Mechanisms of DNA Damage, Repair, and Mutagenesis. *Environmental and Molecular Mutagenesis*, 235- 63.

Cicek et al . (2015). Antioxidant and anti-hemolytic activities of Juniperus oxycedrus L. essential oil. *Journal of Essential Oil Research 27(2)*, 145-152.

Erman et al. (2019). Cell Division, Cytotoxicity, and the Assays Used in the Detection of Cytotoxicity. *Cytotoxicity - Definition, Identification, and Cytotoxic Compounds*. doi:https://doi.org/10.5772/intechopen.88368.

Firbas, Peter, and Tomaž Amon. (2014). Chromosome Damage Studies in the Onion Plant Allium Cepa L. *Caryologia*, 25 -35. doi:https://doi.org/10.1080/00087114.2014.891696

Foladlou et al. (2019). Cytotoxicity: A Review of the Definition, Mechanisms, and Methods. *Journal of Pharmacy and Pharmacology*, 1461-1474. doi:10.1111/jphp.13135

Haddouchi et al. (2016). Screening phytochimique, activités. *Phytothérapie*. doi::10.1007/s10298-016-1086-8

Hasan, et al. (2017). Genotoxicity Testing: Progress and Prospects for the next Decade. *Expert Opinion on Drug Metabolism & Toxicology*, 1089- 98. doi:https://doi.org/10.1080/17425255.2017.1375097

Hastings et al. (2019). DNA damage and repair in human disease. *Nature Reviews Genetics*, 115. doi:10.1038/s41576-019-0146-4

J. M. P. Q. Delić et al. (2018). Tannins: A Review of Their Biological Effects. *Journal of Food Science*, S1445-S1454.

Kacem, K et al. (2010). Diversité des espèces de Juniperus L. (Cupressaceae) dans les régions montagneuses d'Algérie. *Revue d'Ecologie (Terre et Vie)*, 151-162.

Kähkönen et al. (2008). Polyphenolic compounds in berries: A review. *ournal of Agricultural and Food Chemistry*, 4153-4164. doi:10.1021/jf703464k

Kaur et al. (2018). Allium cepa test system for assessing genotoxicity of plant extracts. *Toxicology Reports*. doi:10.1016/j.toxrep.2018.02.006

Kumar et al. (2020). Necrosis vs. Apoptosis: A Review. *International Journal of Molecular Sciences*. doi:10.3390/ijms21051661

Kusumaningrum, Hermin Pancasakti, et al. (2012). Chromosomes and Mitotic Cell Division Phase In Onion Roots After 24 Hours Acetoorcein Soaking Time. *Bioma : Berkala Ilmiah Biologi,*, 46. doi:https://doi.org/10.14710/bioma.14.2.46-48

L. Vilar et al. (2016). *European Atlas of Forest Tree Species.* Récupéré sur European Atlas of Forest Tree Species: https://w3id.org/mtv/FISE-Comm/v01/e013abb

LaCroix et al. (2002). Tannins and astringency: A review. *Food Hydrocolloids*, 254-264. doi:10.1016/j.foodhyd.2008.02.004

Lee et al. (2017). Genotoxicity assays: A review of the methods and their applications. *Mutation Research/Reviews in Mutation Research*. doi: 10.1016/j.mrrev.2017.06.001

Leme, D. M., and Marin-Morales, M. A. (2009). Allium cepa test in environmental monitoring: A review on its application. *Mutation Research/Reviews in Mutation Research*, 71-81. doi:https://doi.org/10.1016/j.mrrev.2009.06.002.

Li et al. (2019). *Journal: Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*.

Li, Weijia, et al. (2015). Study of the in Vitro Cytotoxicity Testing of Medical Devices. *Biomedical Reports,*, 617- 20. doi:https://doi.org/10.3892/br.2015.481

Liu, Z., et al. (2018). Cytotoxicity testing: A review of current methods and their applications. *Journal of Pharmacy and Pharmacology*, 313-324.

López-Romero et al. (2018). Evidence of Some Natural Products with Antigenotoxic Effects. Part 2 Plants, Vegetables, and Natural Resin. *Nutrients*, 1954.

Moreno L et al. (2015). Esplugues J. Pharmacological. *Pharmacol Toxicol*, 2202. doi:1998;82[2]:108-112.

Orhan et al . (2012). Identification of hypoglycaemic . *J Ethnopharmacol* , 110-118.

R. K. Mishra et al. (2019). Cytotoxic agents: A review of their mechanisms of action and clinical applications. *Journal: Journal of Clinical and Translational Research*. doi:10.1177/2165070519843114

Radhika et al. (2019). A review on genotoxicity, its molecular mechanisms, regulatory testing in drug development process. *International Journal of Pharmaceutical Sciences and Research*, 4054-4069.

Scalbert et al. (1991). Antimicrobial properties of tannins. Phytochemistry. *3875-*, 654–658.

Shaibani et al. (2019). Cytotoxicity assays: A review of the methods and their applications. *Journal of Pharmaceutical and Biomedical Analysis*, 10.1016/j.jpba.2019.05.023.

Souza et al. (2018). Comparative analysis of genotoxicity tests using Allium cepa and Tradescantia fluminensis. *Environmental Science and Pollution Research*. doi:10.1007/s11356-018-3264-1

Tang et al. (2015). Antioxidant and antimicrobial activities of Juniperus oxycedrus L. fruits and wood. *Journal of Food Science*, S1344-S1351.

Tsimogiannins. (2006). The contribution of flavonoid C-ring on. *Innovat Food Sci Emerg Tech*, 140-146.

Urquiaga and Leighton. (2000). Phenolic compounds in plants: an overview. *Phytochemistry*, 147-155.

Urquiaga et al. (2000). Plant polyphenol antioxidants and oxidative stress. *Biol.*, 33: 55–64.

X. Wang et al. (2017). Flavonoids and Neuroprotection. *Journal of Agricultural and Food Chemistry*, 341-351.

Yang et al. (2021). Genotoxicity Testing and Recent Advances ». Genome Instability & Disease. *Genome Instability & Disease*, 1–21. doi:oi.org/10.1038/s41586-021-03477-4

Yinping Lei. (2017). Metabolomics of medicinal plants: Current trends and future perspectives. *Journal of Pharmaceutical and Biomedical Analysis*, 123-134. doi:10.1016/j.jpba.2017.03.006