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BELHORI Ahmed

Title

Aristolochia longa L. & *Ephedra alata* Decne: Ethnopharmacology, phytochemistry, cytotoxicity and genotoxicity.

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Intitulé

Aristolochia longa L. & *Ephedra alata* Decne : Ethnopharmacologie, phytochimie, cytotoxicité et génotoxicité.

Devant les membres de jury :

Président	Mr. Toufik BENAISSA	MAA
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الملخص

تُستخدم (*Aristolochia longa*) و (*Ephedra alata*) على نطاق واسع في الطب التقليدي الجزائري لعلاج السرطان واضطرابات البروستات وتنظيم الأيض. ومع ذلك، يثير الاستخدام غير المنضبط وغياب إرشادات الجرعات مخاوف تتعلق بالسلامة. هدف هذه الدراسة إلى توثيق تطبيقاتهما الإثنوفارماكولوجية، وتوصيف ملفاتهما الفيتوكيميائية، وتقييم تأثيراتهما السيتوجينية والسمّية الخلوية باستخدام اختبار (*Allium cepa*).

شمل المسح المنظم 200 من العشّارين والمعالجين التقليديين والمستخدمين المحليين في ثماني مناطق غربية بالجزائر، وجُمعت بيانات عن الأجزاء النباتية وطرق التحضير والجرعات ودواعي الاستخدام العلاجية. أُعدّت مستخلصات مائية وهيدروإيثانولية ومُنقوع لأخشاب (*Aristolochia longa*) وجذور (*Ephedra alata*) ، ثم جُفّفت ووزنت لتحديد عائدات الاستخلاص. كُوّنت مجموع البوليفينولات والفلافونويدات والتانينات بطريقة قياس الامتصاص الطيفي (اختبارات فولين–جيوكال، كلوريد الألمنيوم، والفانيلين).

تم تقييم السّمية الخلوية والجينية عن طريق تعريض الأنسجة الانقسامية لجذور (*Allium cepa*) لمجموعة من تراكيز المستخلصات؛ حيث حُسب مؤشر الانقسام ومؤشرات المراحل ومعدّلات الشذوذات الصبغية.

أكدت البيانات الإثنوفارماكولوجية الاستخدام السائد للنوعين في علاج السرطان، مع تطبيقات ثانوية في صحة البروستات والتحكم في الوزن. أظهرت المستخلصات الإيثانولية كفاءة أعلى في الاستخلاص، إذ أنتجت تركيزات أكبر من البوليفينولات والفلافونويدات والتانينات مقارنةً بالمستخلصات المائية. في اختبار (*Allium cepa*) ، أدّت مستخلصات النباتين إلى تثبيط انقسامات الخلايا بشكل معتمد على التركيز وزيادة في الشذوذات الصبغية. أبدى (*Aristolochia longa*) نشاطاً سيتوتوكسياً وجينوتوكسياً قويين، تمثلان في توقف حاد للانقسام وكثرة التعدد الصبغي، بينما أظهر (*Ephedra alata*) فعالية سيتوتوكسية معتدلة مع دلالات محتملة مضادة للأورام.

إن الفعالية البيولوجية القوية لكلٍّ من (*Aristolochia longa*) و (*Ephedra alata*) تُبرز إمكاناتهما العلاجية، لكنها في الوقت نفسه تبرز مخاطر جينوتوكسية ملحوظة، لا سيما بالنسبة إلى .(*Aristolochia longa*) ولضمان الاستخدام الآمن والفعال لهذه العلاجات التقليدية، ثمة حاجة ملحّة لإجراء دراسات حركية وعلمية سمّية دقيقة، إلى جانب تبنّي بروتوكولات موحدة للجرعات وضبط الجودة.

الكلمات المفتاحية: النباتات الطبية، الإثنوفارماكولوجيا، (Aristolochia longa)، (Ephedra alata)، الكيمياء النباتية، السّمية الخلوية والجينية، .(Allium cepa)

Abstract

Aristolochia longa and *Ephedra alata* are widely used in Algerian traditional medicine for cancer management, prostate disorders, and metabolic regulation. However, uncontrolled usage and lack of dosage guidance raise safety concerns. This study aimed to document their ethnopharmacological applications, characterize their phytochemical profiles, and evaluate their cytogenotoxic effects using the *Allium cepa* bioassay.

A structured survey of 200 herbalists, traditional healers, and local users across eight western Algerian regions captured data on plant parts, preparation methods, dosages, and therapeutic indications. Aqueous, hydroethanolic, and decoction extracts of *A. longa* stems and *E. alata* roots were prepared, dried, and weighed to determine extraction yields. Total polyphenols, flavonoids, and tannins were quantified spectrophotometrically (Folin–Ciocalteu, $AlCl_3$, and vanillin assays). Cytogenotoxicity was assessed by exposing *Allium cepa* root meristems to a range of extract concentrations; mitotic index, phase indices, and chromosomal aberration frequencies were calculated.

Ethnopharmacological data confirmed predominant use of both species for cancer, with secondary applications in prostate health and weight control. Ethanol extracts demonstrated superior extraction efficiency, yielding notably higher concentrations of polyphenols, flavonoids, and tannins than aqueous preparations. In *Allium cepa* assays, both plant extracts induced a concentration-dependent inhibition of cell division and an increase in chromosomal aberrations. *A. longa* exhibited the strongest cytotoxic and genotoxic activity, characterized by marked mitotic arrest and frequent polyploidy, while *E. alata* showed moderate cytotoxicity coupled with antitumor potential.

The potent bioactivity of *A. longa* and *E. alata* underscores their therapeutic promise but also highlights significant genotoxic risks, particularly for *A. longa*. Rigorous pharmacokinetic and toxicological investigations, coupled with standardized dosing and quality-control protocols, are essential to ensure the safe and effective application of these traditional remedies.

Key words: Medicinal plants, Ethnopharmacology, *Aristolochia longa*, *Ephedra alata,* phytochemistry, Cytogenotoxicity, *allium cepa.*

Résumé

Aristolochia longa et *Ephedra alata* sont largement employées dans la médecine traditionnelle algérienne pour la prise en charge du cancer, les troubles de la prostate et la régulation métabolique. Cependant, leur usage non contrôlé et l'absence de recommandations posologiques soulèvent des problèmes de sécurité. Cette étude visait à documenter leurs usages ethnopharmacologiques, à caractériser leur profil phytochimique et à évaluer leurs effets cytogénotoxiques à l'aide du test *Allium cepa*.

Une enquête structurée auprès de 200 herboristes, praticiens traditionnels et usagers locaux, répartis dans huit régions de l'Ouest algérien, a permis de recueillir des données sur les parties de plantes utilisées, les modes de préparation, les dosages et les indications thérapeutiques. Des extraits aqueux, hydroéthanoliques et de décoction des tiges *d'A. longa* et des racines *d'E. alata* ont été préparés, séchés et pesés afin de déterminer les rendements d'extraction. Les polyphénols totaux, les flavonoïdes et les tanins ont été quantifiés par spectrophotométrie (tests de Folin–Ciocalteu, $AlCl_3$ et vanilline). La cytogénotoxicité a été évaluée en exposant des méristèmes racinaires d'*Allium cepa* à différentes concentrations d'extraits ; l'indice mitotique, les indices de phase et les fréquences d'aberrations chromosomiques ont été calculés.

Les données ethnopharmacologiques confirment un usage prédominant des deux espèces pour le cancer, avec des applications secondaires en santé prostatique et contrôle du poids. Les extraits éthanoliques ont montré une efficacité d'extraction supérieure, avec des teneurs nettement plus élevées en polyphénols, flavonoïdes et tanins que les préparations aqueuses. Dans les essais sur *Allium cepa*, tous les extraits ont induit une inhibition de la division cellulaire dépendant de la concentration et une augmentation des aberrations chromosomiques. *A. longa* a présenté l'activité cytotoxique et génotoxique la plus marquée, caractérisée par un arrêt mitotique prononcé et une polyploïdie fréquente, tandis qu'*E. alata* a montré une cytotoxicité modérée associée à un potentiel antitumoral.

La puissante bioactive *d'A. longa* et *d'E. alata* souligne leur promesse thérapeutique mais met également en lumière des risques génotoxiques significatifs, en particulier pour *A. longa*. Des investigations pharmacocinétiques et toxicologiques rigoureuses, associées à des protocoles standardisés de dosage et de contrôle qualité, sont indispensables pour garantir l'usage sûr et efficace de ces remèdes traditionnels.

Mots-clés : Plantes médicinales · Ethnopharmacologie · *Aristolochia longa* · *Ephedra alata* · Phytochimie · Cytogénotoxicité · *Allium cepa*

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Dedications

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Miloud

Dedications

We dedicate this thesis to our dear parents, who have always been by our side and supported us throughout these long years of study.

To my brother and supporter, Ouss who encouraged me on my educational tour.

To all of our friends, particularly those with whom we worked in the laboratory.

To our professor, Mr. TAIBI Khaled,

To all members of the Molecular and Cellular Biology Group.

And to all those who value hard work and never back down in the face of life's challenges.

Sidahmed

Abbreviation's list

IUCN: International Union for conservation of nature

STAPF: Refers to Otto Stapf (1857–1933), an Austrian-born British botanist who first described the subspecies.

Batt. & Trab: Denotes Jules Aimé Battandier (1848–1922) and Louis Charles Trabut (1853–1929), French botanists who contributed to the classification of North African flora.

DNA: Deoxyribonuleic acid

MI: Mitotic index

CAs: Chromosomal aberrations

GAE: gallic acid equivalent

GE: quercetin equivalent

TA: tannic acid

MMS: methyl methane sulfonate

CN: negative control

CP: Positive control

PYD and DPD: pyridinoline and deoxypyridinoline

AAs: aristolochic acids

HLs: human lymphocytes

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State of the Art

State of the Art

Traditional medicine serves as a crucial foundation for scientific discovery, guiding research towards novel therapeutic avenues. Medicinal plants represent a vital and enduring source of potential treatments for diverse diseases. The knowledge encompassing their therapeutic applications is predominantly transmitted orally across generations, unique to specific populations or ethnic groups (Benarba et al., 2021).

In Algeria, where medicinal plants have been utilized for centuries, this traditional knowledge retains profound significance and the country recognized as one of the Arab world's biodiversity hotspots with 3,164 documented plant species, nevertheless exhibits a notable gap: ethnobotanical research remains relatively limited, where local communities continue to depend heavily on traditional healers for primary healthcare. Critically, populations frequently utilize these botanicals with little to no documented scientific understanding of their potential toxicity or appropriate dosage. This widespread reliance on traditionally used plants, despite the absence of established safety profiles, underscores a critical research gap and significant public health concern. Consequently, the imperative for rigorous cytogenotoxicity testing is paramount to scientifically evaluate the safety of these remedies. Therefore, the present study aims to assess the ethnopharmacology uses, phytochemicals properties and the cytogenotoxic of selected medicinal plants which are: *Ephedra alata Decne, Aristolochia longa L* using the Allium cepa assay.

1. Ethnopharmacology

Ethnopharmacology is the study of how modern and indigenous societies view and use plants. The use of natural products with healing properties is as old as human civilization, and for centuries, minerals, animal products, and plants have been the primary sources of drugs. Several active compounds have been discovered from plants on the basis of ethnopharmacology information and used either directly or as lead compounds for patented medications (Boudjelal et al., 2013). A large number of articles in ethnopharmacology have been identified and analysed. Over the past 50 years, the field has evolved from being conventionally linked to “traditional knowledge,” drug discovery, and certain areas of pharmacology to playing an increasingly prominent role in disease prevention (notably within food science). This analysis

also underscores how research priorities have shifted toward addressing the needs and interests of rapidly developing economies (Yeung et al., 2018).

This research, grounded in extensive ethnopharmacological surveys conducted over the past decade and enriched by rigorously verified records from a systematic literature review, has resulted in the creation of a comprehensive biodiversity database of medicinal plants (Vyas *et al.,* 2019).

2. *Ephedra alata Decne*

Ephedra alata Decne belongs to the family *Ephedraceae* (genus: *Ephedra*) (Bell et Bachman 2011). Commonly known as "alenda," it is widely distributed across the Algerian Sahara and throughout North Africa (Algeria, Egypt, Libya, Mauritania, Morocco, Tunisia, and Western Sahara) and is listed as a threatened species on the IUCN Red List (Bell et Bachman, 2011; Berreghioua et Ziane, 2025).

Figure 1. General View of *Ephedra alata* subsp. alenda (STAPF) Batt. & Trab A: Overall appearance of the plant, B: Flowering branch, C: Fully bloomed flower (Hadjadj et al., 2020)

In March 2023, multiple specimens were collected at Boukais (south of Béchar, southwestern Algeria) and authenticated by local herbalists. A recent ethnobotanical investigation demonstrated that the plant extracts, particularly the methanolic extract, contain bioactive

compounds namely alkaloids, flavonoids, tannins, and terpenes which impart strong antioxidant activity and inhibit pathogenic bacterial growth (Berreghioua et Ziane, 2025). According to an ethnopharmacological study, this plant is used in Algeria to treat cancers, including breast, brain, colorectal, liver, and lung cancers (Taïbi et al., 2020).

3. *Aristolochia longa L.*

Aristolochia longa L. is a perennial herbaceous species in the family *Aristolochiaceae*, locally known in Algeria as "bereztem" or "berrostom," and is characterized by its tuberous root system. The genus *Aristolochia* comprises over 500 species distributed worldwide, especially in tropical and subtropical regions, situating *A. longa* within a broadly recognized group of medicinally important plants (Lamari et Negache, 2021). In Algeria, *A. longa* is widely distributed, with specimens from Médéa (west of Algiers) collected for biochemical characterization that revealed the presence of various lipid compounds in roots and aerial parts (Cherif et al., 2009), rhizomes from Taourirt Aden (Kabylia region) harvested for mineral element analysis, which underscored a distinctive trace element profile relevant to its medicinal applications (Lamari et Negache, 2021); ethnobotanical surveys in Sétif (eastern Algeria) documenting local uses for skin and gastrointestinal ailments (Merouani *et al.,* 2020); and investigations in Mascara (western Algeria, Tissemssilet) focusing on ethnomedicinal practices and phytochemical properties of roots collected in March 2009 (Benarba et Meddah, 2014). Phytochemical analyses have identified methyl esters and various fatty acids in essential oils extracted from stalks, rhizomes, and leaves (Cherif *et al.,* 2009), while screenings for secondary metabolites detected polyphenols, flavonoids, saponins, terpenoids, and alkaloids compounds that likely underpin the plant's antioxidant and antimicrobial activities (Merouani *et al.,* 2020). Ethnopharmacologically, traditional healers across these regions use *A. longa* extensively: approximately 43 % of reported applications involve cancer treatment primarily using powdered roots (and occasionally leaves) administered orally to target breast, bone, digestive, prostate, ovarian, and uterine malignancies (Merouani *et al.,* 2020; Taïbi *et al*., 2020); 17 % address diabetes management via consumption of root or leaf powders to ameliorate glycemic symptoms (Merouani *et al*., 2020); 12 % entail treatment of cattle wounds through topical application of crushed or powdered rhizome (Merouani *et al.,* 2020); and the remaining 16 % target gastrointestinal disorders 9 % for intestinal ailments and 7 % for stomach conditions—where powdered plant material is ingested, often mixed with water or honey (Merouani *et al.,* 2020). The root remains the most frequently used plant part, prepared as a fine powder for oral

administration or as a poultice for topical use, depending on the specific ailment (Benarba et Meddah, 2014).

Figure 2. Stages of germination and the formation of *Aristolochia longa L.* tuber (Merouani *et al,* 2020).

Figure 3. *Aristolochia longa* L. (Source : https://www.inaturalist.org/photos/1691943).

4. Cyto-genotoxicity

Cytotoxicity, defined as the intrinsic capacity of chemical or biological agents to induce damage or cell death by disrupting membrane integrity, perturbing essential metabolic pathways, or

triggering programmed cell death (apoptosis), constitutes a fundamental endpoint in both pharmacological efficacy assessments and toxicological safety evaluations, as it directly reflects an agent's therapeutic potential against target cells as well as its collateral risk to non-target tissues (León *et al.*, 2021).

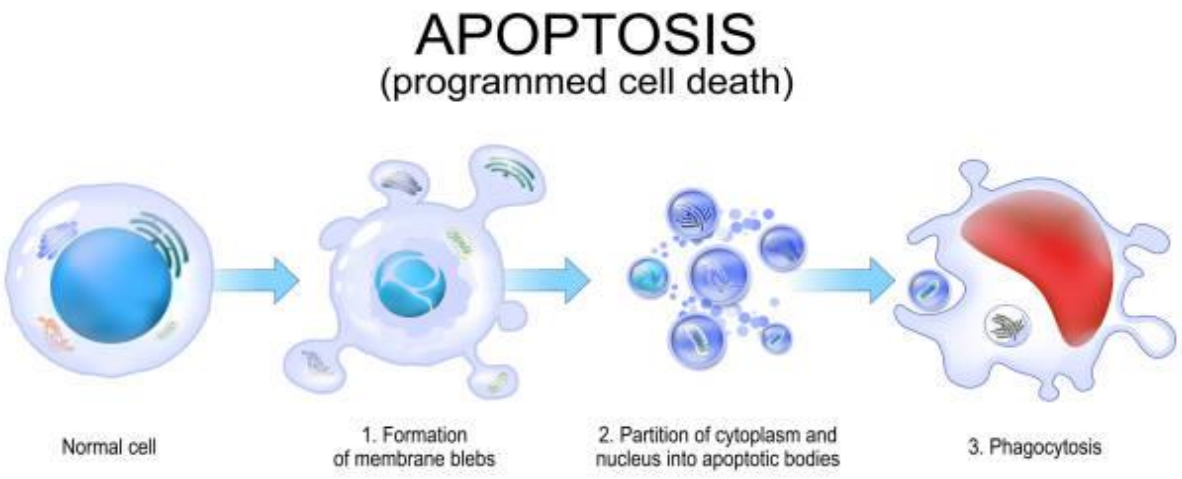

Figure 4. apoptosis (programmed cell death)

Source : https://www.istockphoto.com/search/2/image-film?phrase=cell+death

Apoptosis, necrosis and ferroptosis represent distinct modalities of cell death that together ensure tissue integrity and organismal homeostasis, apoptosis is a tightly regulated, gene-driven process whereby caspases orchestrate the orderly dismantling and removal of aging, damaged or superfluous cells without eliciting inflammation, thus preventing aberrant cell accumulation (Bocsci, 2024). by contrast, necrosis is an unprogrammed, passive form of cell demise triggered by severe insults such as hypoxia, toxins or trauma, characterized by organelle swelling, plasma-membrane rupture and uncontrolled release of intracellular contents that provoke secondary inflammation and collateral tissue injury (Galluzzi et al., 2018). More recently, ferroptosis has been defined as an iron-dependent, non-apoptotic pathway driven by the catastrophic build-up of lipid peroxides and unique metabolic vulnerabilities, distinguishing it biochemically and pathologically from both apoptotic and necrotic death programs (Dixon et al., 2012)

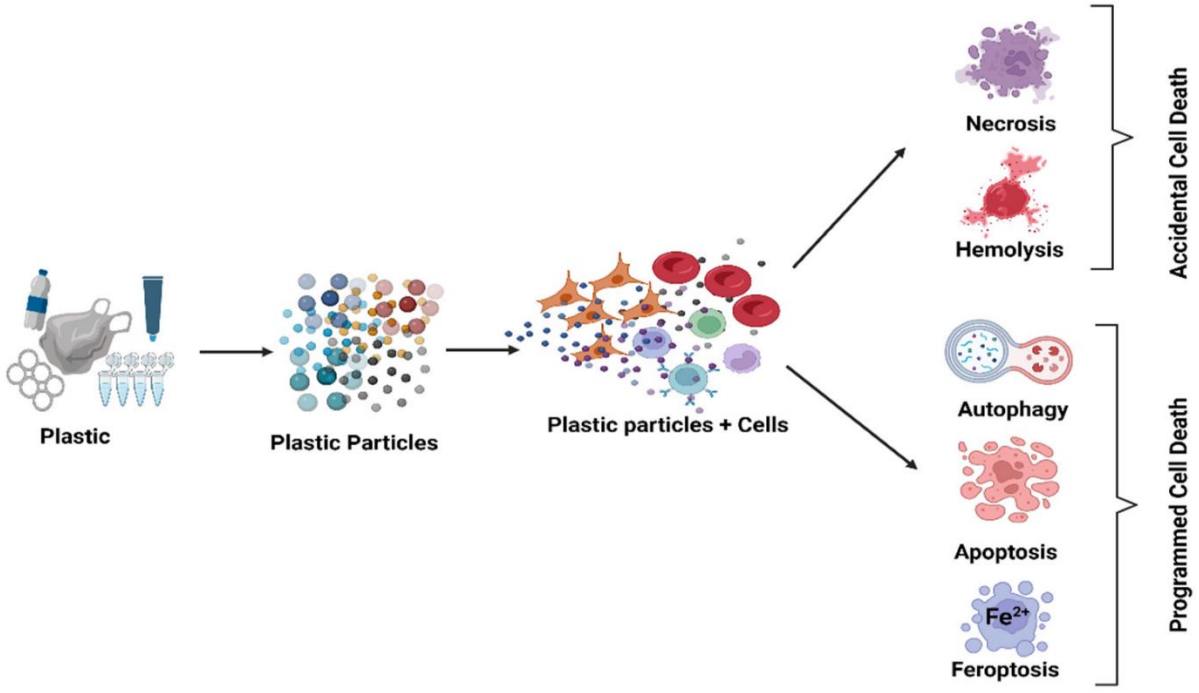

Figure 5. Important Factors Affecting Induction of Cell Death, Oxidative Stress and DNA Damage by Nano- and Microplastic Particles In Vitro (Kamil et al., 2024)

In parallel, genotoxicity describes the ability of various agents including synthetic chemicals, ionizing radiation, and certain biological toxins to inflict direct or indirect damage upon cellular DNA, whether through base substitutions, strand breaks, cross-linking, or chromosomal mis segregation, thereby fostering mutational events that underpin carcinogenesis and heritable genetic disorders (Brusick, 2009). Together, cytotoxicity and genotoxicity represent complementary yet distinct dimensions of bioactivity: the former gauges immediate cellular viability impacts, while the latter assesses longer-term genomic integrity risks, making them indispensable, interrelated parameters in environmental monitoring, drug development pipelines, and regulatory safety frameworks.

5. Cell anomalies and aberrations

Cell anomalies and chromosomal aberrations are structural changes in chromosomes—such as deletions, duplications, or insertions that can disrupt genes and lead to developmental disorders and congenital diseases. These changes may be inherited or arise spontaneously and, although less frequent than small DNA mutations, their ability to alter gene function makes them important targets in genetic diagnostics and research (Kloosterman et al., 2014)

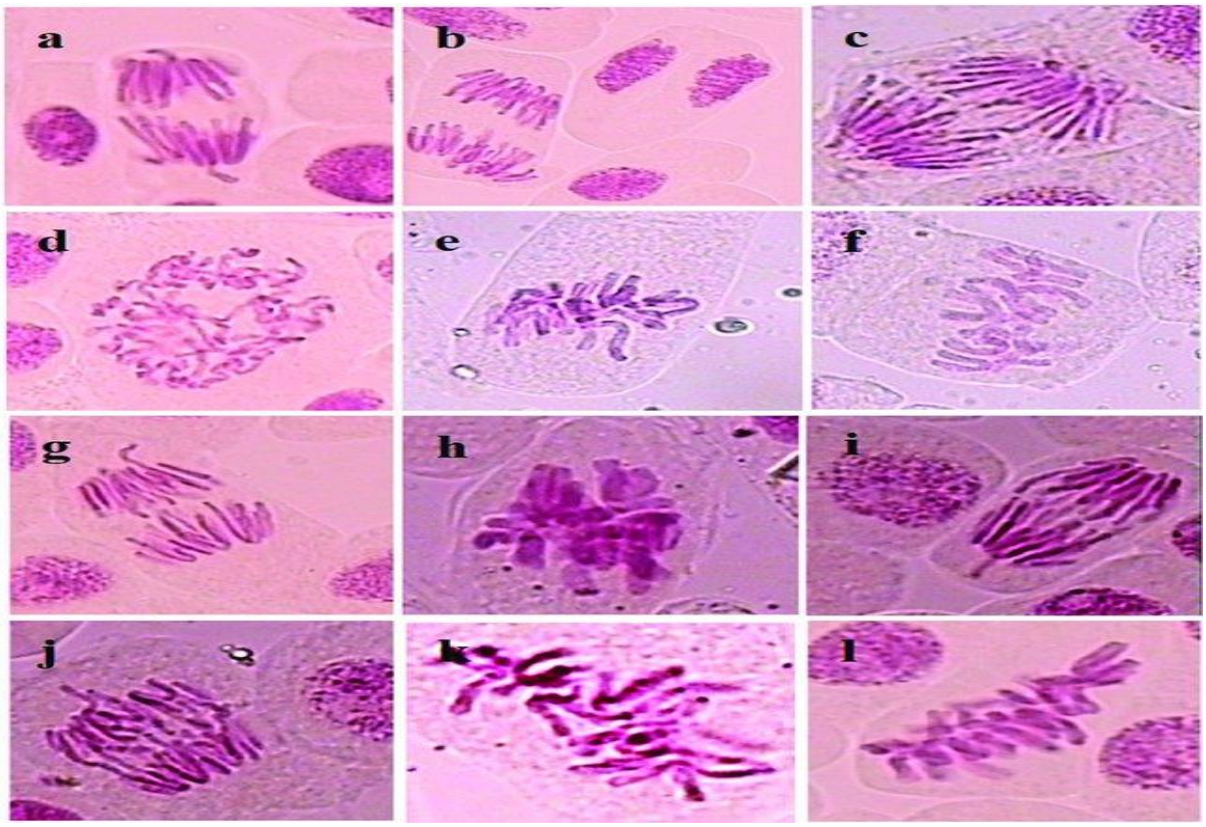

Figure 6. Different types of chromosomal aberrations induced by textile wastewater in Allium cepa root tips: Vagrant chromosome (a), Multipolar anaphase and binucleated cell (b); Anaphase bridge (c); Spindle disturbance in anaphase (d); Disturbed metaphase (e, f); Multipolar anaphase with vagrant chromosome (g); Stickiness (h); Anaphase bridge with vagrant chromosome (i); Anaphase with micro bridge (j); C-Mitosis (k, l) (Khan *et al.*, 2019)

6. Allium cepa

The *Allium cepa* test is a practical and reliable method used to detect the toxic and DNA-damaging effects of industrial wastewater by observing changes in onion root growth, cell division, and chromosome structure. It helps identify harmful substances in treated effluents, even when those substances are within legal environmental limits (Pathiratne *et al.,* 2015).

Higher plants are recognized as excellent genetic models for detecting environmental mutations and are frequently used in ecological monitoring studies. *Allium cepa* has been employed to assess DNA damage among plant species. The use of *A. cepa* as a test system to detect mutations dates back to the 1940s and continues to this day to evaluate many chemical agents, contributing to its wider application in environmental monitoring. *Allium cepa* offers the advantages of being a low-cost, easily handled assay with benefits over other short-term tests that require prior preparation of samples (Naf'i et Khalil., 2022).

As part of (Molecular Biology Group) study on the acquisition and valorisation of bioactive plant extracts from local raw materials under sustainable conditions, the assessment of the genetic safety of extracts using an eco-compatible method represents an important step. From this perspective, the present research aims to evaluate the biosafety potential of *Aristolochia longa L*. and *Ephedra alata Decne*, extracts obtained under different conditions, by identifying the mitotic index (MI) and chromosomal aberrations (CAs) using a sustainable method. Moreover, the application of fast and efficient physicochemical methods will allow for the comparison of the phytochemical profiles of the investigated plant extracts. Therefore, this study was undertaken to evaluate the comparative cytotoxic and genotoxic effects of aqueous and hydroalcoholic extracts and powder of the medicinal plants selected *Aristolochia longa* and *Ephedra alata* using the *Allium cepa* assay.

Methodology

Methodology

1. Ethnopharmacological study

An ethnopharmacological survey was conducted to collect traditional knowledge regarding the medicinal use of two selected plants *Aristolochia longa* and *Ephedra alata*. A structured questionnaire was administered to several individuals from different regions in Algeria, including herbalists, traditional healers, and local users, in regions where these plants are commonly used. The questionnaire focused on identifying different diseases treated by these plants, the plant parts used, preparation methods (such as decoction, infusion, and maceration), quantities and dosages, frequency and duration of use, therapeutic indications, and any possible side effects. The data that were collected through face-to-face interviews, phone calls and social media, the local language when needed to ensure clarity and accuracy. All responses were treated confidentially and used exclusively for academic research. The collected data were compiled and analysed using descriptive statistics in Microsoft Excel to determine common usage patterns and support the experimental design of cytotoxicity and genotoxicity assessments.

2. Preparation of plant extracts

cleaned and dry *Ephedra alata* roots and *Aristolochia longa* stems were bought from an herbalist, and, after that, they were crushed in an electric blinder into a fairly soft powder that was stored in a well-covered jar and kept out of light.

The aqueous extract was prepared through macerating 50 g of each plant powder in 500 mL of distilled water in a glass flask. The mixture was left with stirring at room temperature in the dark for 24 hours. Theextract was then filtered using Whatman paper, and the obtained filtrate was dehydrated in an incubator at 40 °C to obtain a dry residue, which was then stored in a clean box sheltered from light.

The ethanolic extract was prepared by putting 50 g of powder in a glass flask containing 500 mL ethanol 70 %. The mixture was agitated and macerated for 24 hours. The resulting extractwas filtered using Whatman paper, and the filtrate was dehydrated in an incubator at 40°C to obtain a dryresidue, which was then stored in a tidy box out of the light.

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.

2.1 Preparation of decoction

Dried and powdered plant material from *Aristolochia longa* and *Ephedra alata* was used for the preparation of decoctions. For each concentration (2.5, 5, 10, and 20 mg/mL), the appropriate amount of powder was weighed and mixed with distilled water. The mixtures were brought to a boil and maintained at boiling for 15 minutes. After decoction, the solutions were allowed to cool to room temperature. Each preparation was then transferred to clean bottles and covered with aluminium foil to protect it from light (Figure.7).

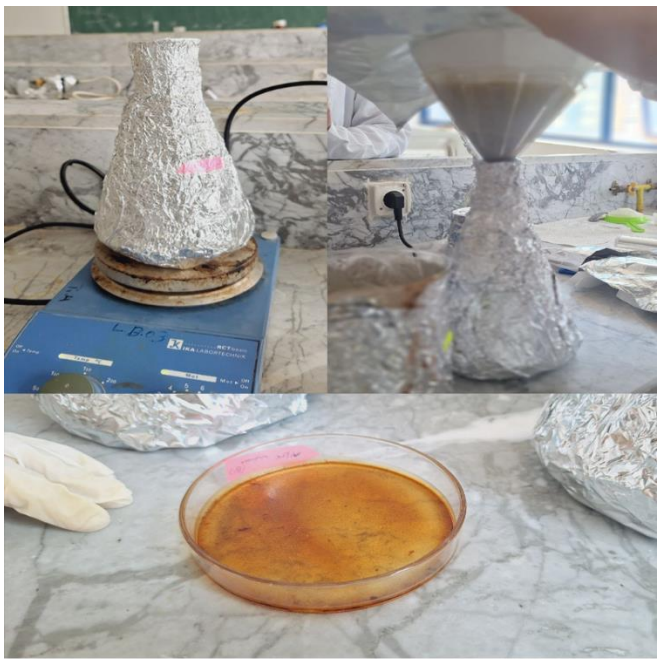

Figure 7. Extracts plants preparation

3. Phytochemical compounds

3.1. Polyphenols

A volume of 200 µL of each extract (aqueous and ethanolic) with different concentrations (0.2 ,0.5,1,1.5 and 2mg/mL), and other powder concentrations (2.5,5,10 and 20mg/mL), was added by using a micropipette 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 roomtemperature. 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. Then, the absorbance is measured at 765 nm. The total polyphenol content is derived from the calibration curve, and the findings are expressed as gallic acid equivalent (mg GAE/g) of the extract. Each experiment was replicated at least three times.

3.2. Flavonoids

In an Eppendorf tube, 1 mL of each extract was mixed with 1 ml of Aluminium chloride (AlCl3) 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 (mg QE)/g extract. Each experiment was repeated of three times a minimum.

3.3. Tannins

A volume of 37.5 µL of each extract was added to 1125 µL of 49% vanillin in methanol, and the mixture was vigorously mixed. Then, 562.5 µL of concentrated hydrochloric acid (HCl) was added. The reaction mixture was left to stand at room temperature for 20 minutes. Absorbance was measured at 550 nm against a blank. The total tannin content was determined using a standard calibration curve, and results were expressed as mg tannic acid equivalent (mg TA/g) of extract. Each experiment was conducted at least three times (Figure.8).

Figure 8. Spectrophotometer.

4. Mitotic index analysis (*allium cepa* essay)

The onion plant (*A. cepa*, 2n = 16), a species belonging to the Alliaceae family, was utilized for the present study. Uniform-sized bulbs were procured from a local market in Tiaret, Algeria. After carefully removing the outer peel and existing roots, the bulbs were washed thoroughly with distilled water. To prepare them for the *Allium cepa* assay, the outer scales and the desiccated basal plate were removed without damaging the root primordia. Once the roots reached the minimum of length (2 cm), the bulbs were selected for analysis. Each germinated bulb was positioned in a test tube containing distilled water, ensuring that only the root region was submerged.

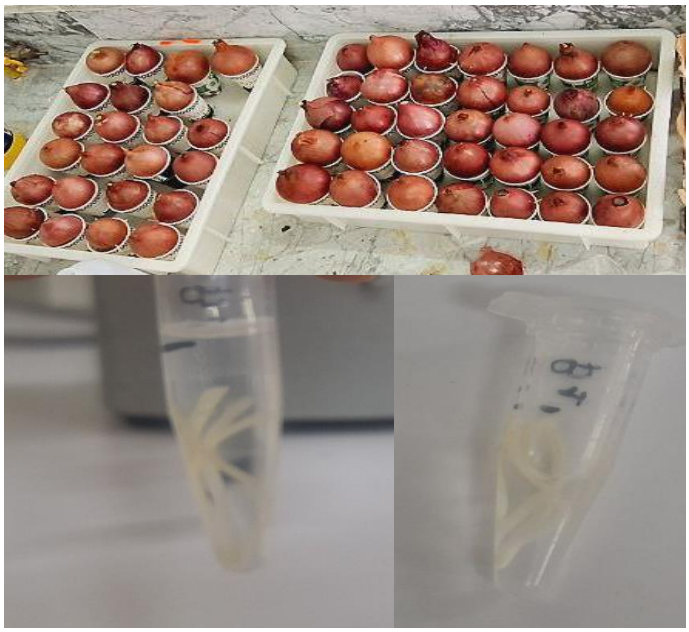

Figure 9. Procedures of A. cepa test.

Following the initial growth phase, the four most vigorously developing onion bulbs were selected and exposed for 24 hours to varying concentrations of each plant extracts and also the powder, prepared in both aqueous and ethanolic solutions and powder solutions. The selected concentrations were informed by findings from previous ethnopharmacological investigations into the traditional medicinal applications of each plant in Algeria, as reported by Taïbi et al. (2021) (Figure.9).

After 24 hours of exposure under dark conditions, approximately the terminal 2 cm of each root tip was excised for cytological analysis. The collected root segments were immediately fixed in Carnoy's fixative at 4 °C for a night at least to preserve cellular structures. For long-term storage, the fixed samples were transferred to 70% ethanol. Prior to microscopic examination, the roots underwent acid hydrolysis using 1 N hydrochloric acid, a standard procedure to soften tissues and enhance dye uptake. Subsequently, the samples were stained with Feulgen reagent specific for DNA while protected from light to prevent degradation of the stain.

After treatment, the roots were counted and the length of the roots in each bulb was measured. And the mitotic zones were immersed in a drop of 45% acetic acid on a clean slide and squashed under a cover glass. In order to spread the cells evenly on the surface of the slide, squashing was accomplished with a bouncing action by striking the cover glass with a match stick. Approximately 5000 to 6000 cells were scored for each concentration and control. MI were expressed in terms of divided cells/total cells and the chromosomal aberrations were expressed in terms of aberrant cells/divided cells. A statistical analysis was performed on the collected data (Figure.10).

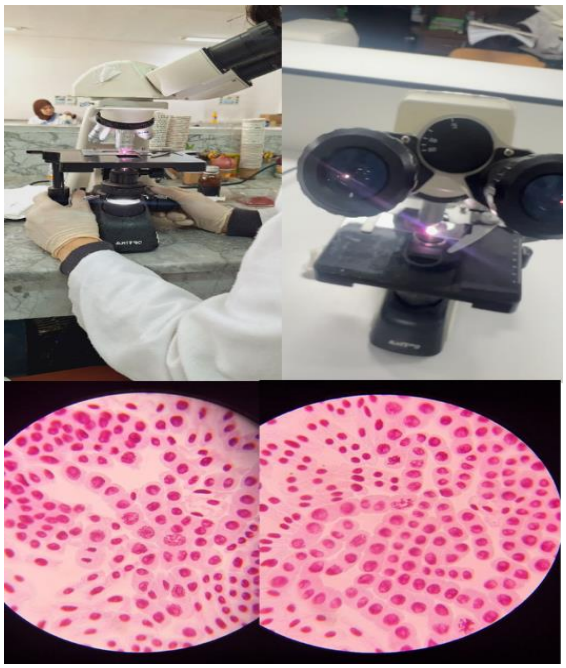

Figure 10. Observation of mitotic phases and aberrations.

The mitotic index is a measure of how many cells are undergoing mitosis in a group of cells. It is calculated by dividing the number of cells in mitosis by the total number of observed cells, then multiplying the result by 100.

The Phase Index (PI) represents the percentage of dividing cells that are in a particular phase of mitosis. It is calculated by dividing the number of cells in a specific mitotic phase by the total number of dividing cells, then multiplying the result by 100.

The Chromosomal Aberrations Index (CAs) represents the percentage of dividing cells that show structural or numerical chromosomal abnormalities. It is calculated by dividing the number of cells with chromosomal aberrations by the total number of dividing cells, then multiplying the result by 100.

Results

The study's findings are structured into three complementary sections. First, the ethnopharmacological survey uncovers traditional applications through field interviews. Second, the phytochemical profiling characterizes the plant's major bioactive constituents via laboratory assays. Third, the cytotoxicity and genotoxicity assessments of the two plants *Aristolochia longa* and *Ephedra alata.*

1. Ethnopharmacology

A structured questionnaire was administered to 200 individuals from different regions in Algeria (Tiaret, Sougeur, Oued Souf, Oran, mehdia, El Bayadh, Bechar, Media).

1.1. Respondents' information

The ethnopharmacological survey analysis reveals a notable correlation between participant age and the prevalence/transmission of "repetitions" (interpreted as frequencies of use or knowledge of traditional practices). Intermediate age groups ([37-49] years old) stand out with the highest frequencies (approximately 41-42 repetitions), suggesting their central role in holding and disseminating this knowledge. Conversely, extreme age groups, the youngest ([0-18] years old) and the oldest ([56-61] years old), show significantly lower frequencies (approximately 8 repetitions), which could indicate limited acquisition or an erosion of this knowledge within these cohorts. The observed distribution generally describes an increase in repetitions from youth towards middle adulthood, followed by a decrease (Figure.11).

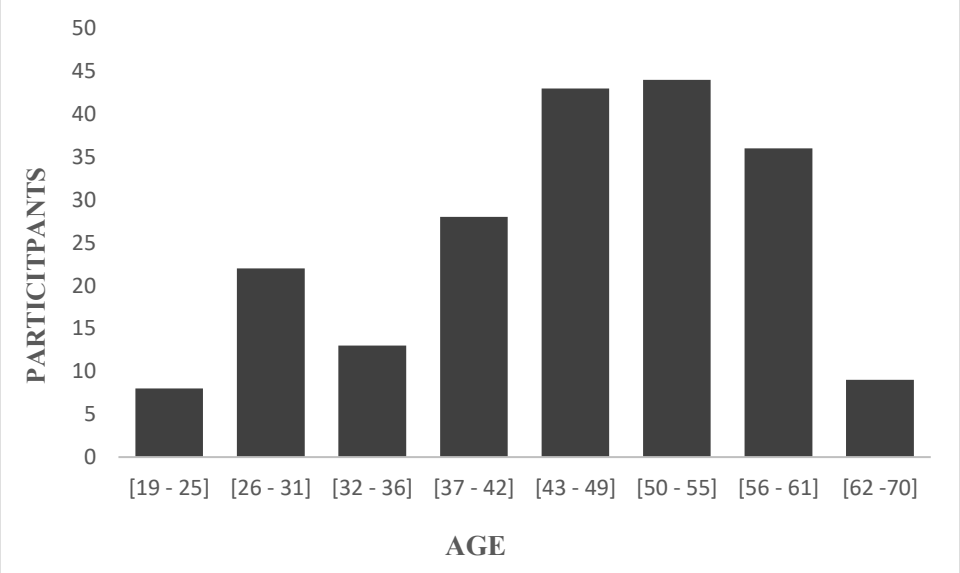

Figure 11. Distribution of the age groups of the participants.

Men occupied 98% of the respondent, otherwise, the rest is female (Figure.12).

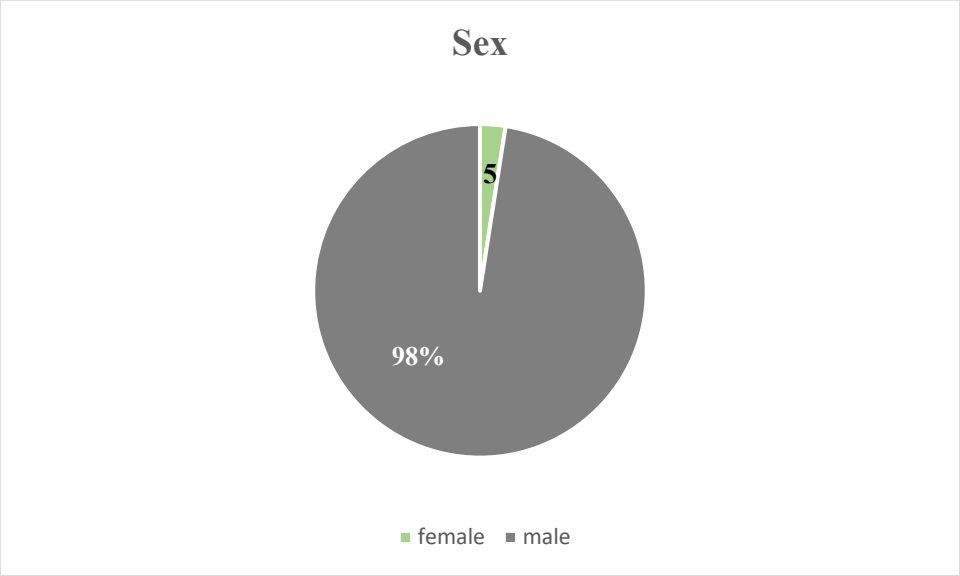

Figure 12. Distribution of the sex groups of the participants.

This chart shows that the vast majority of respondents have only a middle-school education (about 103 participants), followed by those with a high-school level (47). Far fewer are illiterate (22) or have just primary schooling (17), and only a small fraction holds an academic (university) qualification (14). In other words, over half of the sample stopped their formal education at or before high school, with very few reaching higher-education levels (Figure.13).

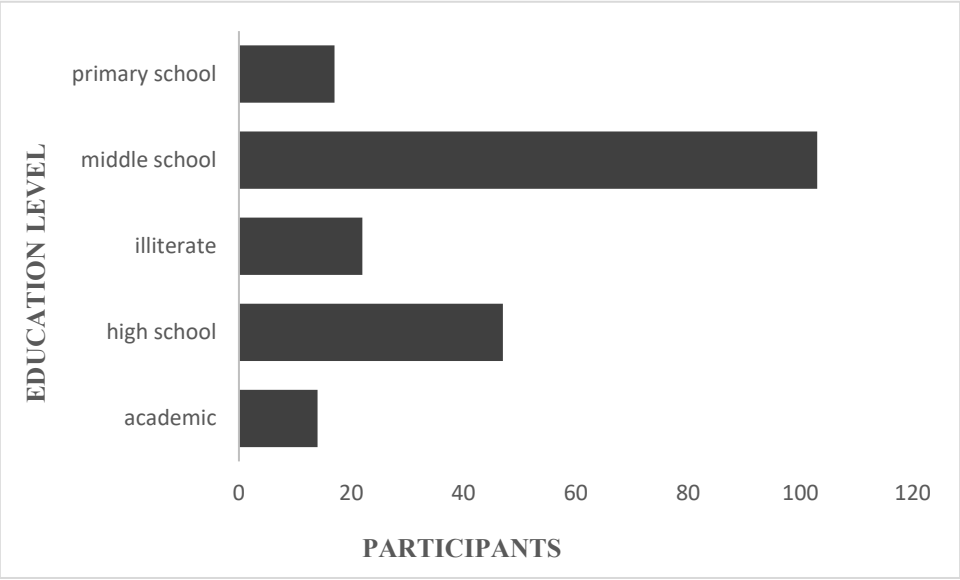

Figure 13. study level of the participants

Almost all participants (94 %) live in urban areas, with only a small minority (6 %) coming from rural settings (Figure,14).

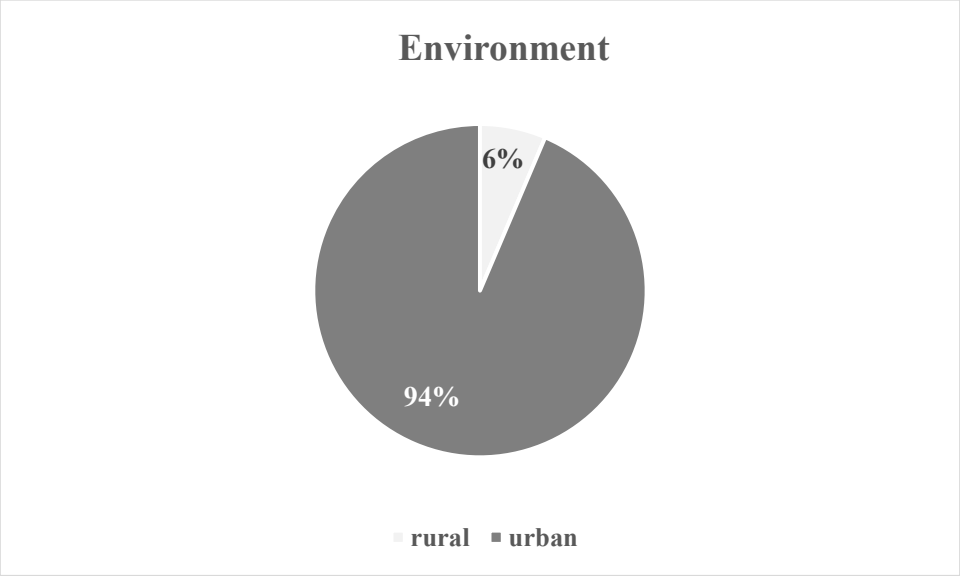

Figure 14. Distribution of participants according to their living environment.

Out of 203 participants, an overwhelming 168 (≈82.8 %) identified as herbalists. The next largest groups were patients, with 10 respondents (≈4.9 %), and the unemployed, at 7 individuals (≈3.4 %). Housewives accounted for 5 participants (≈2.5 %), while students and teachers each contributed 3 (≈1.5 % apiece), and retirees numbered 2 (≈1.0 %). Finally, doctors, firemen, municipal workers, policemen, and seamstresses were each represented by a single respondent (≈0.5 % each) (Figure.15)

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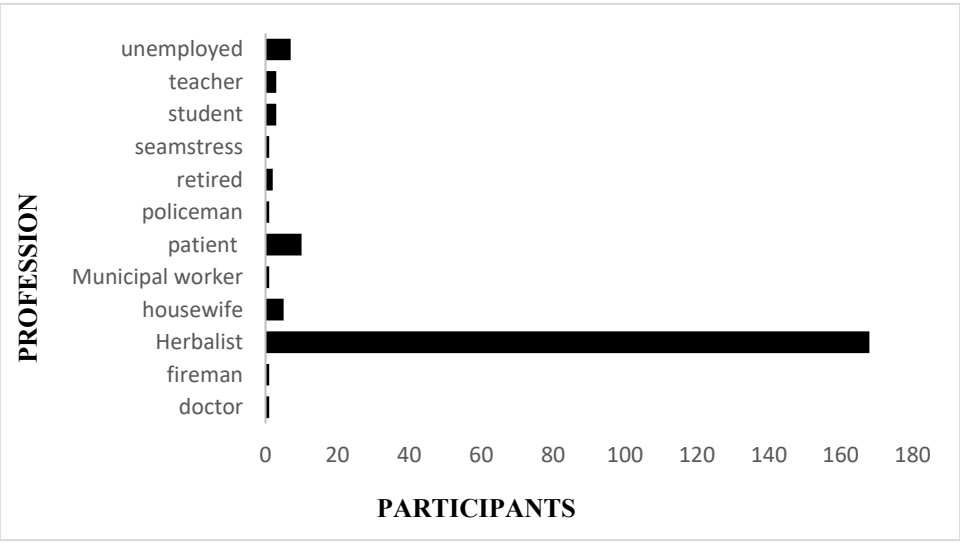

Figure 15. Nature of the function of the participants.

The number of mentions for cancer treatment stands out massively among the therapeutic uses, reaching approximately 135-140 citations. This represents the most pronounced effect and by far the most frequently cited category among all listed ailments. In contrast, for stomach ulcers, panic attacks, microbial infections, general infections, hemorrhoids, thyroid gland disorders, diarrhea, cysts, cholesterol, and cardiovascular conditions, the number of citations is very low, generally less than 5, and even around 2 to 3 citations for many of these specific ailments. Regarding prostate problems, a slightly higher frequency is noted, reaching approximately 25-28 citations. While this is significantly higher than the other minor conditions, it remains very far from the prominence of cancer. Thus, despite the presence of a variety of therapeutic uses for different conditions, the application related to cancer clearly remains dominant, concentrating almost all mentions, while other uses are marginal (Figure.16)

Cancer treatment overwhelmingly dominates the reported therapeutic uses, garnering approximately 118-120 citations, making it the most frequently cited category. In stark contrast, conditions such as respiratory issues, microbial infection, fertility problems, digestive issues, and cardiovascular ailments receive very few mentions, generally less than 5 each (around 2-3). Anemia is also minimally cited, with 5-7 mentions. Weight loss, however, stands out as the second most cited use, with approximately 25-27 citations, followed by prostate problems at about 15-17 citations. While these are more prominent than other minor conditions, they remain significantly less cited than cancer. Therefore, cancer-related applications are unequivocally central, with weight loss as a distant secondary focus, and other therapeutic uses being largely marginal (Figure.17)

The graph clearly demonstrates that the utilization of preparation methods is predominantly focused on powder with honey. This method accounts for 48.77% of the recorded mentions, totalling 99 occurrences. Infusion and decoction follow in importance, with 21.67% (44 occurrences) and 16.26% (33 occurrences) respectively. The use of herbal blend and mixed with butter methods is significantly less prevalent, accounting for only 6.90% (14 occurrences) and 6.40% (13 occurrences). This distribution attests to a pronounced specialization in "powder with honey" preparation within the studied practices, while other methods represent secondary uses (Figure.18)

The graph clearly demonstrates that the utilization of preparation methods is predominantly focused on decoction. This method accounts for an overwhelming 82% of the recorded mentions, totalling 166 occurrences. Infusion follows in importance, with 8% (16

occurrences). The use of powder mixed with honey and powder mixed with olive oil methods is significantly less prevalent, accounting for 5% (10 occurrences) and 5% (11 occurrences) respectively. This distribution attests to a pronounced specialization in decoction preparation within the studied practices, while other methods represent secondary uses (Figure.19)

This figure represents information collected by the participants concerning the toxicity of *A. longa*, the most side effect repeated was nausea by cited 110, the plant was also mentioned toxic for pregnant women, the rest of the results was distributed with diarrhea cited 4, kidney cited 10 times, liver 6 times, nosebleed cited 3 times, vomiting cited 6 times (Figure.20).

This figure represents information collected by the participants concerning the toxicity of *E. alata*, the plant hadn't that much as side effect and it can be used by most of all people, it is mentioned that it is toxic for pregnant women, children under 14 years old, and the only side effect that was mentioned is the increase of the rate heart (Figure.21).

2. Phytochemical study

2.1. Evaluation of the yield of extraction

The ethanolic extract yield of *Aristolochia longa* was higher than the aqueous extract yield. Therespective recorded values are 17.23% and 13.25%.

Table 1. Yield of the plant extracts *Aristolochia longa*.

Extract	Yield %
Aqueous extract	13.25%
Ethanolic extract	17.23%

The ethanolic extract yield of *ephedra alata* was also higher than the aqueous extract yield. The respective recorded values are 14.76% and 7.84%

Table 2. Yield of the plant extracts *ephedra alata*.

Extract	Yield %
Aqueous extract	7.84%
Ethanolic extract	14.76%

2.2.Evaluation of the phytochemical compounds

2.2.1. Total phenolics content

a. *Ephedra alata*

The examination of the data reveals a notable difference in the total phenolic content in aqueous and ethanolic plant extracts in the various studied concentrations (Figure.22)

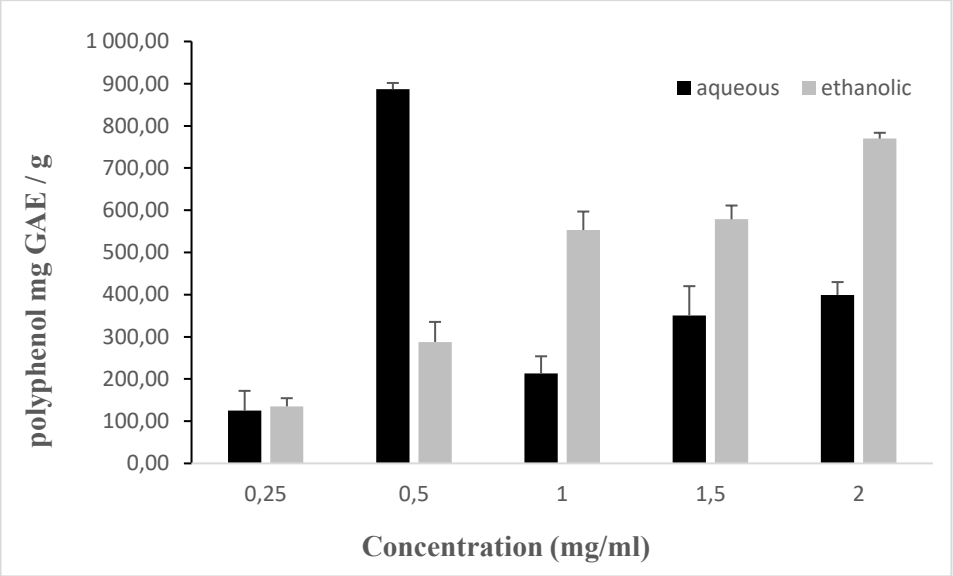

Figure 22. Variation of total phenolic contents in the aqueous and ethanolic extracts of *E. alata*

In general, ethanolic extract marked the higher content of total phenolic content than aqueous extract, except in the second concentration of 0,5 mg/ml, The content of phenolic compounds ranged between a higher value of 769.81± 13.71 mg GAE/g, in the treatment with 2 mg/mL of ethanolic extract, and a higher value of 886.85± 14.83 mg GAE/g , in the treatment with 0,5 mg/mL of the aqueous extract and a lower value of 124.81±47.12 mg GAE/g, in the treatment with 0,25 mg/mL of the aqueous extract.

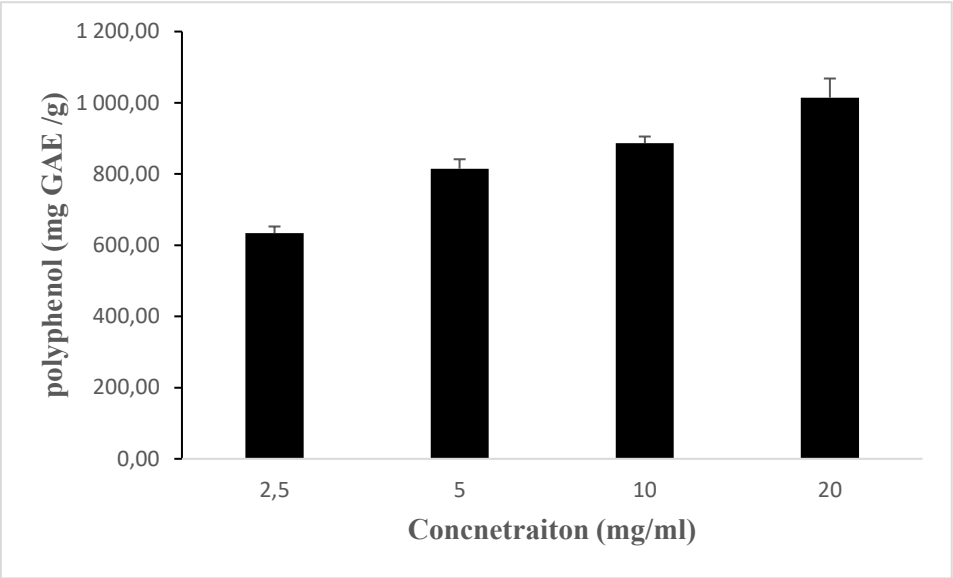

Figure 23. Variation of total phenolic contents in decoction powder of *E. alata*

On the other hand, the content of phenolic compounds ranged between a higher value of 1014.07± 54.07 mg GAE/g, in the treatment with 20 mg/mL, and a lower value of 633.89±18.62 mg GAE/g, in the treatment with 2.5 mg/mL in decoction powder (Figure.23)

b. ***Aristolochia longa***

The examination of the data reveals a notable difference in the total phenolic content in aqueous and ethanolic plant extracts in the various studied concentrations (Figure.24)

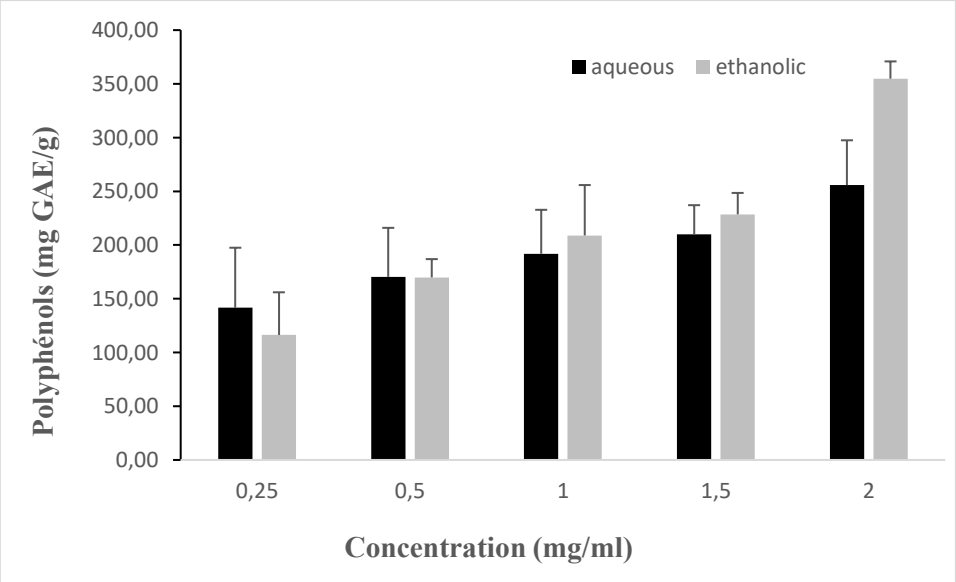

Figure 24. Variation of total phenolic contents in the aqueous and ethanolic extracts of *A. longa*

In general, ethanolic extract marked the higher content of total phenolic content than aqueous extract, except in the two first concentrations of 0,25 mg/ml and 0.5 mg/ml, The content of phenolic compounds ranged between a higher value of 354.81± 70.01 mg GAE/g, in the treatment with 2 mg/mL of ethanolic extract, and a lower value of 116.30±39.72 mg GAE/g, in the treatment with 0,25 mg/mL of the ethanolic extract, and 141.67±55.84 mg GAE/g in the treatment with 0,25 mg/mL of the aqueous extract.

On the other hand, the content of phenolic compounds ranged between a higher value of 832.59± 15.01 mg GAE/g, in the treatment with 20 mg/mL, and a lower value of 218.52±19.96 mg GAE/g, in the treatment with 2.5 mg/mL in decoction powder (Figure.25).

2.2.2. Total flavonoids content

a. Ephedra alata

The analysis of the data reveals a notable difference in the flavonoids content among aqueousand ethanolic plant extracts (Figure.26).

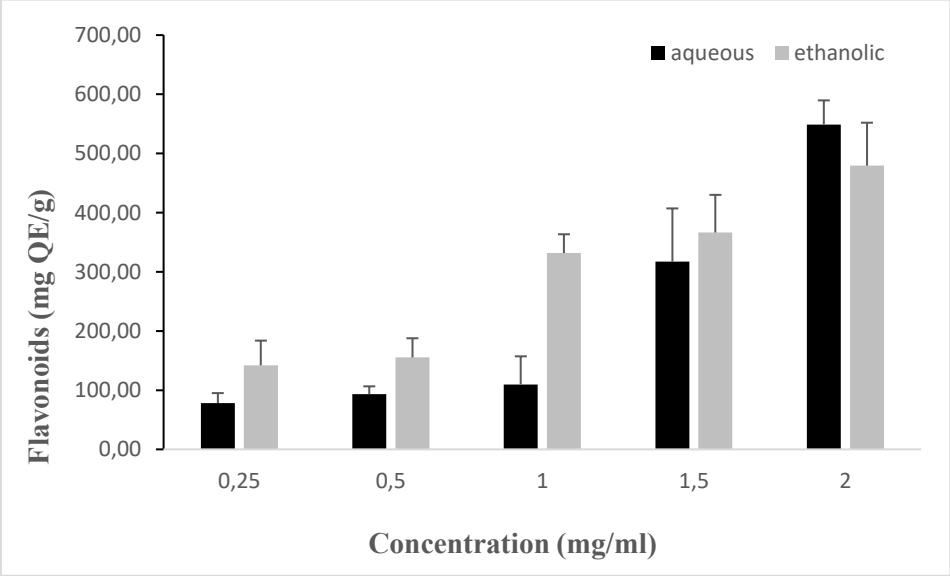

Figure 26. Variation of total flavonoids contents in the aqueous and ethanolic extracts of *E. alata*

The data reveals different values between aqueous and ethanolic extracts, for all first four concentrations results demonstrate that ethanolic extract exhibited a higher content of

total flavonoids except for the last concentration was the opposite, The flavonoids content ranged between 141.94±41.84 mg QE/g in the treatment with 0,25 mg/mL of the ethanolic extract, and 548.89±40.83 mg QE/g in the treatment with 2 mg/mL of the aqueous extract.

On the other hand, the content of flavonoids compounds ranged between a higher value of 1501.39± 15.01 mg QE/g, in the treatment with 20 mg/mL, and a lower value of 218.52±51.55 mg QE/g, in the treatment with 2.5 mg/mL in decoction powder (Figure.27)

b. Aristolochia longa

The examination of the data reveals a notable difference in the total flavonoids content among aqueous and ethanolic plant extracts in the various studied concentrations (Figure.28)

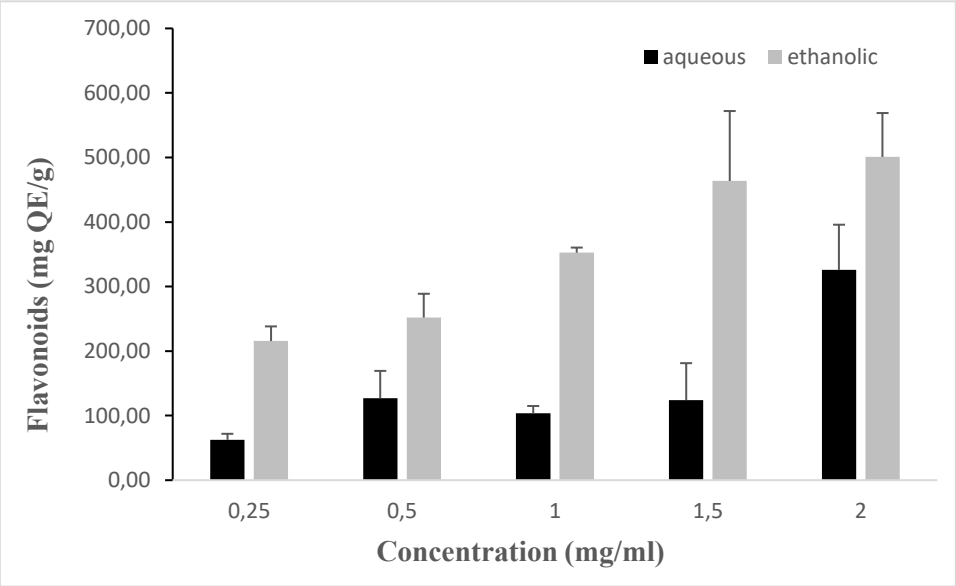

Figure 28. Variation of total flavonoids contents in the aqueous and ethanolic extracts of *A. longa*

The data reveals that the ethanolic extract consistently exhibited a higher flavonoid content than the aqueous extract across all concentrations tested, with flavonoid content ranging between 62.50±9.39 mg QE/g in the aqueous extract at 0.25 mg/mL and 500.83±68.05 mg QE/g in the ethanolic extract at 2 mg/ml.

On the other hand, the content of flavonoids compounds ranged between a higher value of 1165.28± 45.99 mg QE/g, in the treatment with 20 mg/mL, and a lower value of 252.50±7.50 mg QE/g, in the treatment with 2.5 mg/mL in decoction powder (Figure.29)

2.2.3. Total tannins content

a. Ephedra alata

by the same, the content of tannins differs among aqueous and ethanolic extracts (Figure.30)

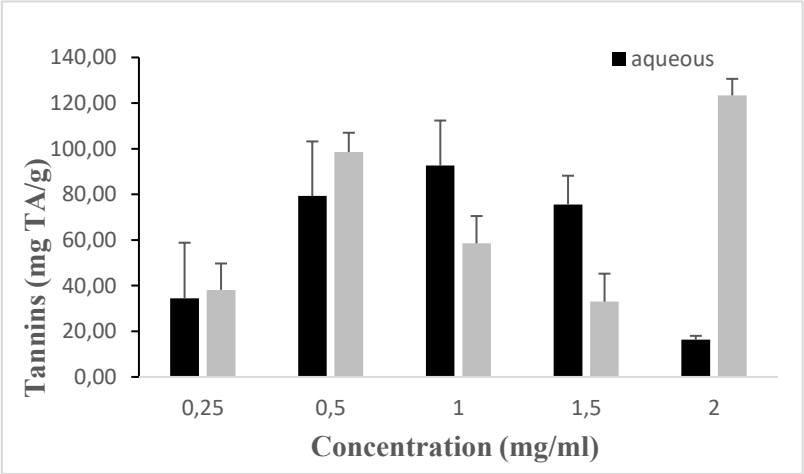

Figure 30. Variation of total tannins contents in the aqueous and ethanolic extracts of *E. alata.*

The data reveals that the ethanolic extract exhibited higher tannin content at 0.25 mg/mL (38.15 mg TA/g), 0.5 mg/mL (98.58 mg TA/g), and 2 mg/mL (123.33 mg TA/g), while the aqueous extract was superior at 1 mg/mL (92.59 mg TA/g) and 1.5 mg/mL (75.56 mg TA/g). Tannin content ranged between 16.30±1.70 mg TA/g in the aqueous extract at 2 mg/mL and 123.33±7.29 mg TA/g in the ethanolic extract at 2 mg/ml.

Results demonstrate a low total tannins content specifically, at 2.5 mg/mL, approximately 12 mg TA/g. This value remains largely unchanged at 5 mg/mL, However, a considerable increase is observed at 10 mg/mL, where the tannin content jumps to approximately 160 mg TA/g. This upward trend culminates at the highest tested concentration of 20 mg/mL, where the tannin content peaks at approximately 265 mg TA/g (Figure.31)

b. Aristolochia longa

The examination of the data reveals a notable difference in the total tannins content among aqueous and ethanolic plant extracts in the various studied concentrations (Figure.32)

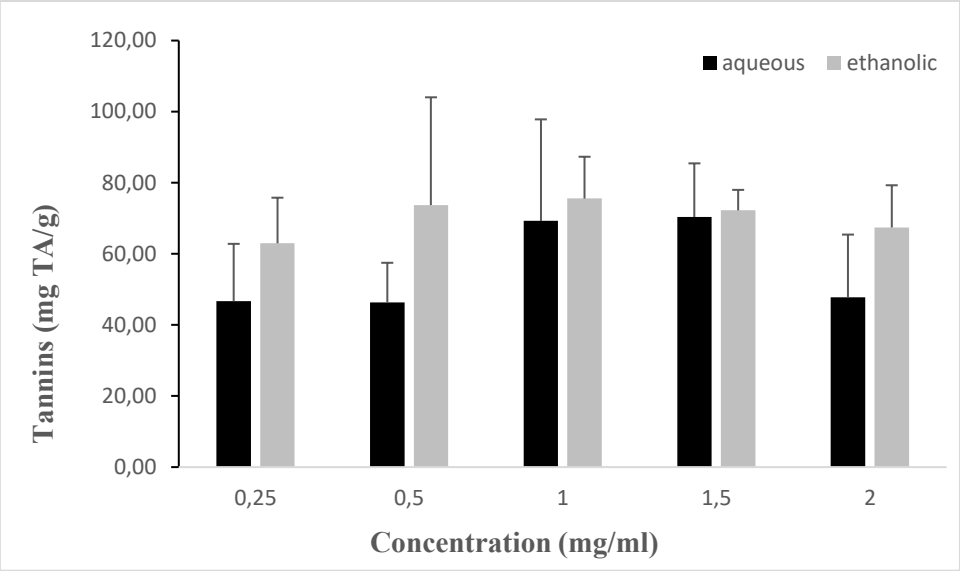

Figure 32. Variation of total tannins contents in the aqueous and ethanolic extracts of *A. longa*

The data reveals different values between aqueous and ethanolic extracts, with the ethanolic extract exhibiting higher tannin content at all five concentrations. Tannin content ranged between 46.30 mg TA/g in the treatment with 0,5 mg/mL of the aqueous extract and 75.56 mg TA/g in the treatment with 1 mg/mL of the ethanolic extract.

The data reveals a generally increasing trend in tannin content with higher extract concentrations, despite a slight decrease at 5 mg/ml, Tannin content ranged between 77.04 mg TA/g in the treatment with 5 mg/mL and 114.07 mg TA/g in the treatment with 20 mg/ml (Figure.33).

3. Evaluation of the cytotoxicity

3.1.Root morphometry

3.1.1. Roots number

The variation in the number of *Allium cepa* roots was measured for each treatment, before and after the incorporation into the extract solutions and the decoction of the plant powders *Aristolochia longa* and *Ephedra alata.*

a. Aristolochia longa:

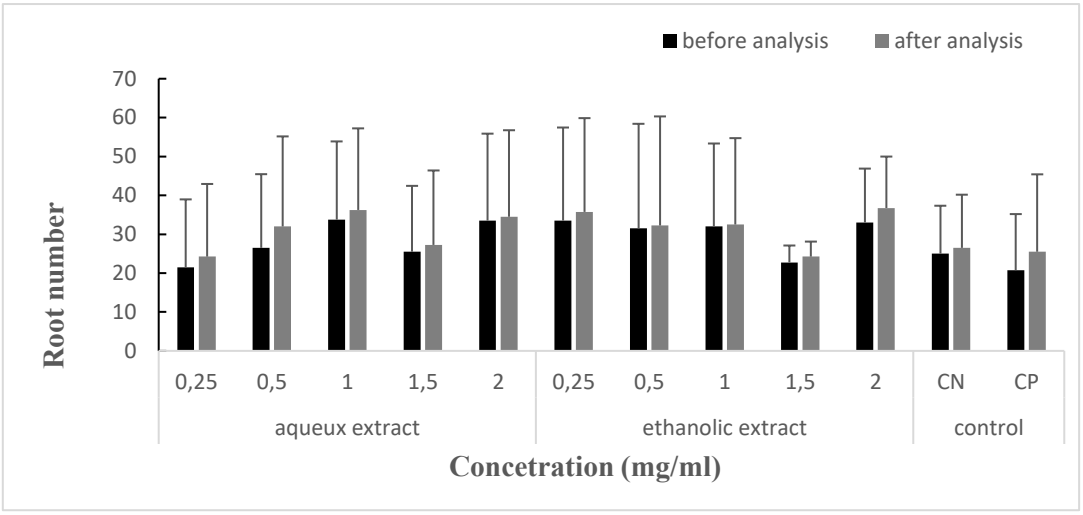

Figure 34. Roots number before and after treatment with plant extracts of *A. longa*.

For the aqueous extract, controls (CN: initial 25 roots; CP: initial 20.75 roots) and all concentrations exhibited increased root counts post-treatment. Before treatment, the maximum mean root count was observed at 1 mg/mL (33.75 roots), while the minimum was at 0.25 mg/mL (21.5 roots). After treatment, the highest root count remained at 1mg/mL (36.25 roots), with CP retaining the lowest final count. In the other hand, the ethanolic extract showed pre-treatment maxima at 0.25 mg/mL (33.5 roots) and minima at 1.5 mg/mL (22.75 roots). Post-exposure, 2 mg/mL achieved the highest final count (36.75 roots), while 1.5 mg/mL remained the lowest (24.25 roots). Both extracts universally stimulated root growth, but displayed distinct progression patterns, ethanolic demonstrated its strongest growth at low concentrations (0.25 mg/mL), and exhibited maximal progression at 2 mg/ml (Figure.34)

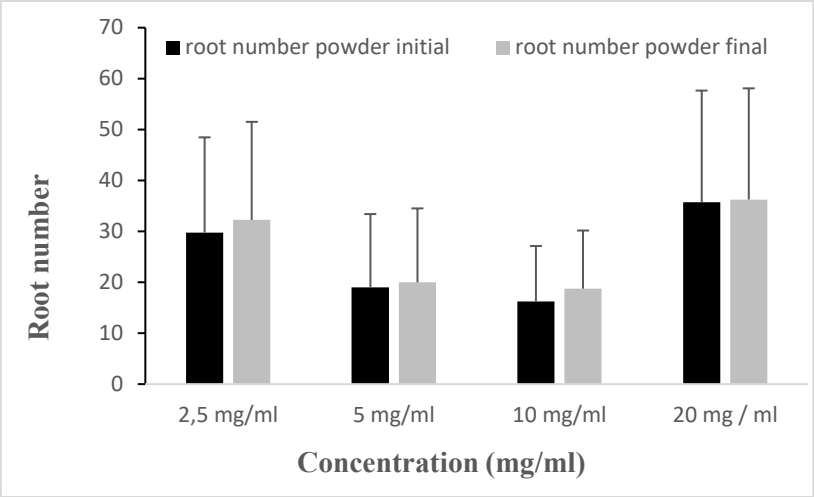

Figure 35. Roots number before and after treatment with plant powder of *A. longa.*

Before treatment, the highest root count occurred at 20 mg/mL while the lowest was observed at 10 mg/ml. After treatment, all concentrations exhibited increased root numbers, with the highest final count maintained at 20 mg/mL and the lowest final count remaining at 10 mg/ml. The most substantial root progression occurred at 2.5 and 10 mg/mL (showing the greatest gain from initial baseline), while the smallest progression was observed at 20 mg/ml. Critically, every concentration demonstrated increased root counts post-treatment, confirming consistent growth stimulation across all tested concentrations of the powder extract (Figure.35)

b. Ephedra alata:

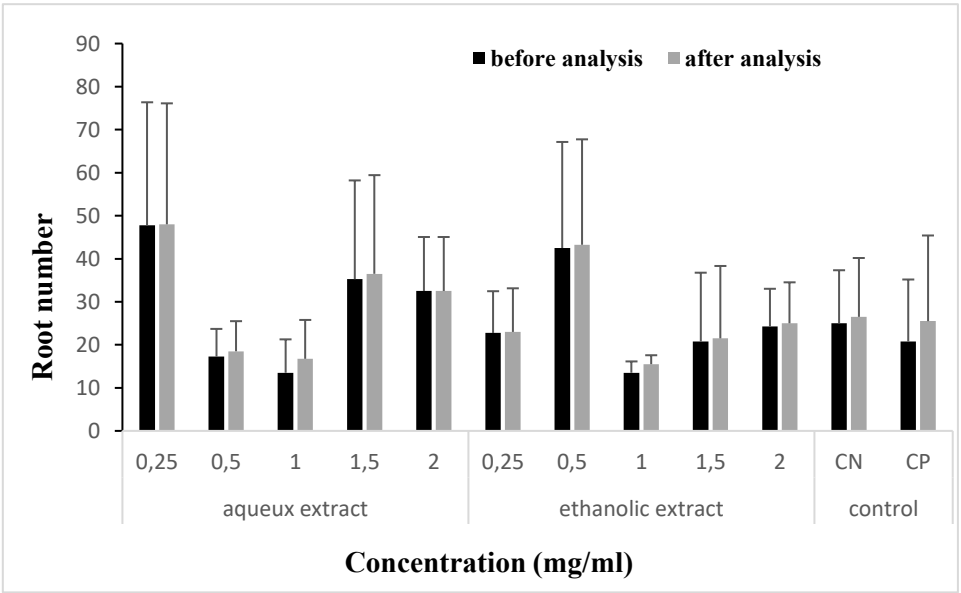

Figure 36. Roots number before and after treatment with plant extracts of *E. alata.*

In the positive control, the number of roots increase after the addition of the MMS solution, ranging from 20 to 26 roots. In contrast, a slight increase in the number of roots is observed in the negative control (in distilled water), from 25 to nearly 27.

Regarding the aqueous and ethanolic extracts, a slight increase in the number of roots is noted at certain concentrations, but without a clear dose-dependent trend. The aqueous extract shows its maximum performance (root gain) at 1 mg/mL (increased by 3.25 roots), while the ethanolic extract reaches its peak at 0.5 mg/mL (43.25 roots). The differences observed between the initial and final values remain small for most concentrations, indicating a moderate effect (Figure.36).

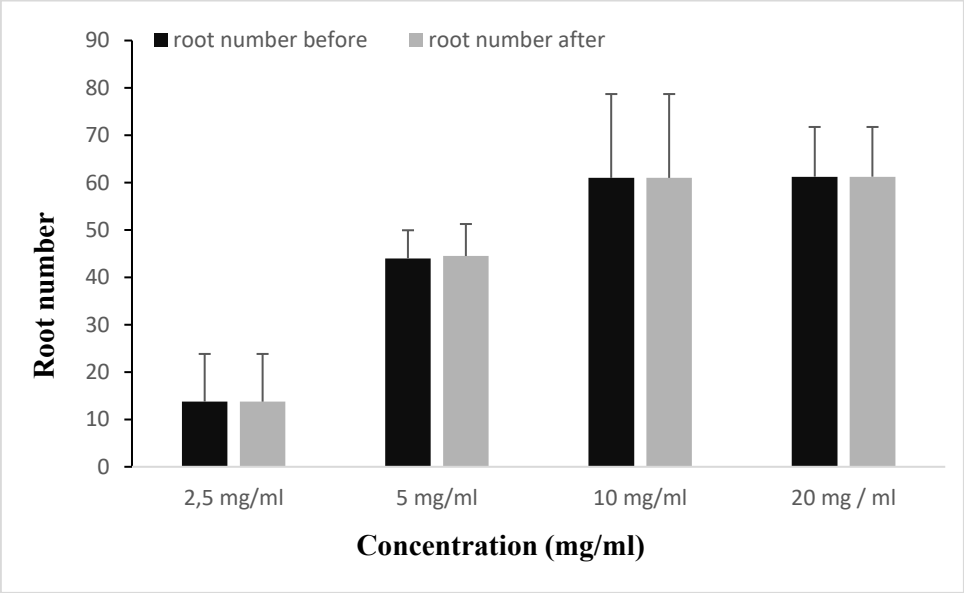

Figure 37. Roots number before and after treatment with plant powder *E. alata*

Pre-treatment root counts were relatively uniform across concentrations, with the highest initial count observed at 20 mg/ml (61.25) and the lowest at 2.5 mg/ml (13.75). Post-treatment, just one concentration had a root proliferation, with the increase occurring at 5 mg/mL (reaching the highest final count). While the rest of the concentrations remained constant with no increase in root number (Figure.37).

3.1.2. Roots length

Similarly, roots length has been measured before and after the addition of plant extracts.

a. Aristolochia longa:

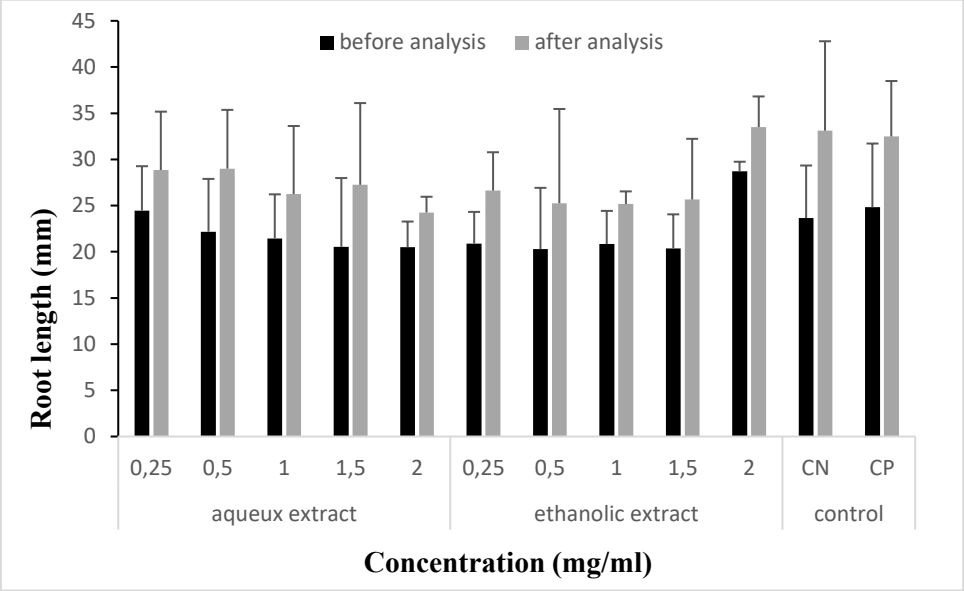

Figure 38. Roots length before and after treatment with plant extracts of *A. longa*.

In the positive control, the root length increases after the addition of the MMS solution, from 24.8 to 32.4 mm as an increase in the length of roots is observed in the negative control (in distilled water), from 23.6 to nearly 33.1 mm.

Regarding the aqueous and ethanolic extracts, there is an increase in the length of roots is noted at all concentrations, the increase in ethanolic extract was higher than aqueous extract I most concentrations, the peak in length root has been marked at 2mg/ml in ethanolic extract (Figure.38)

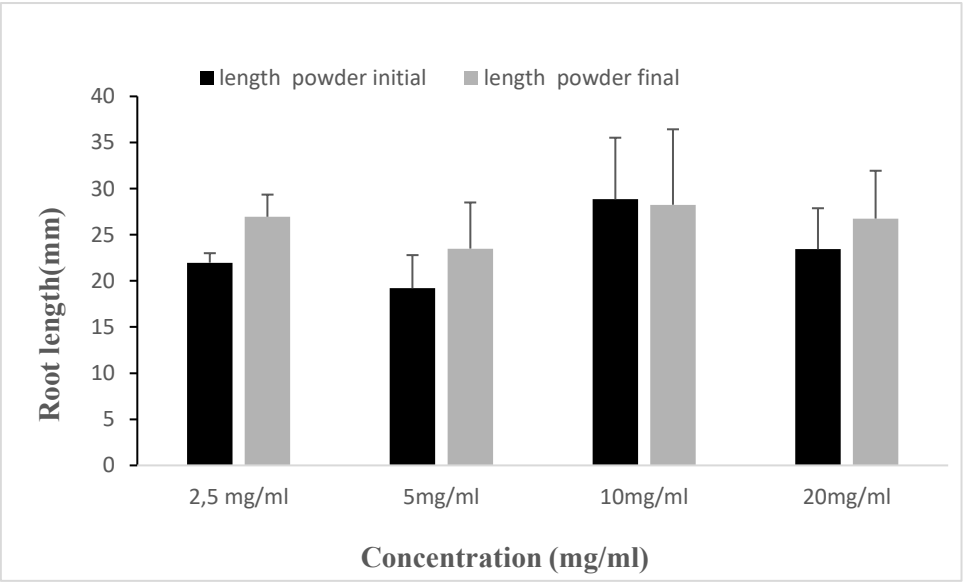

Figure 39. Roots length before and after treatment with plant powder of *A. longa.*

Pre-treatment, the longest roots occurred at 10 mg/mL while the shortest were at 5 mg/mL; post-treatment, 10 mg/mL stayed the longest root but it becomes shorter with about 0.5mm after the extract, 5 mg/mL retained the minimum length despite growth. All concentrations exhibited increased root lengths except for 10 mg/ml showed a decrease in length by 0.5mm, the strongest progression was at 2.5 mg/mL (optimal response) (Figure.39)

a. Ephedra alata:

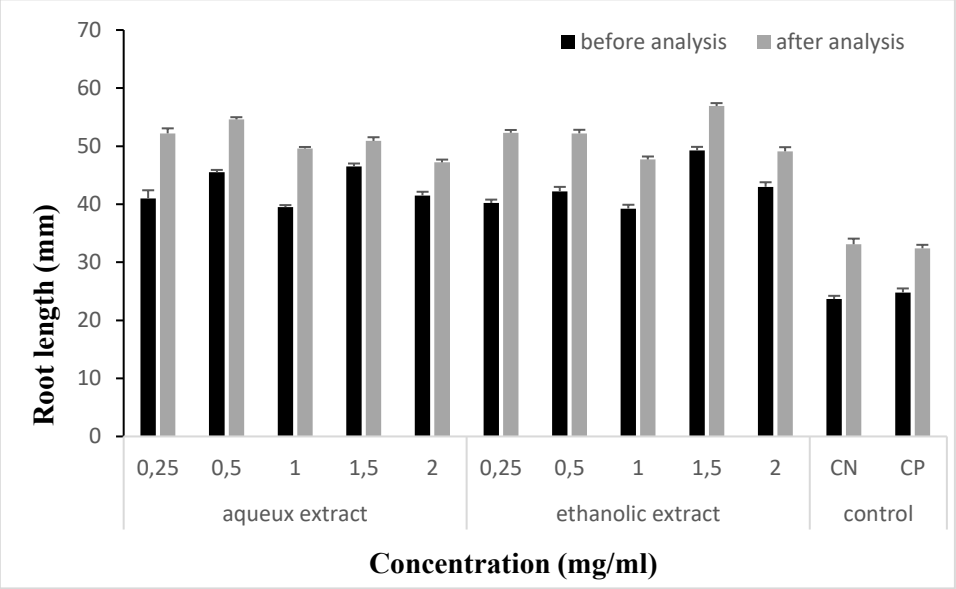

Figure 40. Roots length before and after treatment with plant extracts of *E. alata*.

Aqueous Extract: Before treatment, the maximum root length occurred at 1.5mg/mL, while the minimum was at 1 mg/ml. After treatment, peak length shifted to 0.5 mg/mL and 2 mg/ml was the shortest in aqueous concentrations. All aqueous-treated roots exceeded the positive and negative control (CP and CN)

Ethanolic Extract: Pre-treatment, the greatest length was observed at 1.5 mg/mL and the shortest at 1 mg/ml. post-treatment, 1.5 mg/mL remained the maximum length, while 1 mg/mL was the minimum length. Ethanolic roots similarly surpass CP and CN's length root (Figure.40).

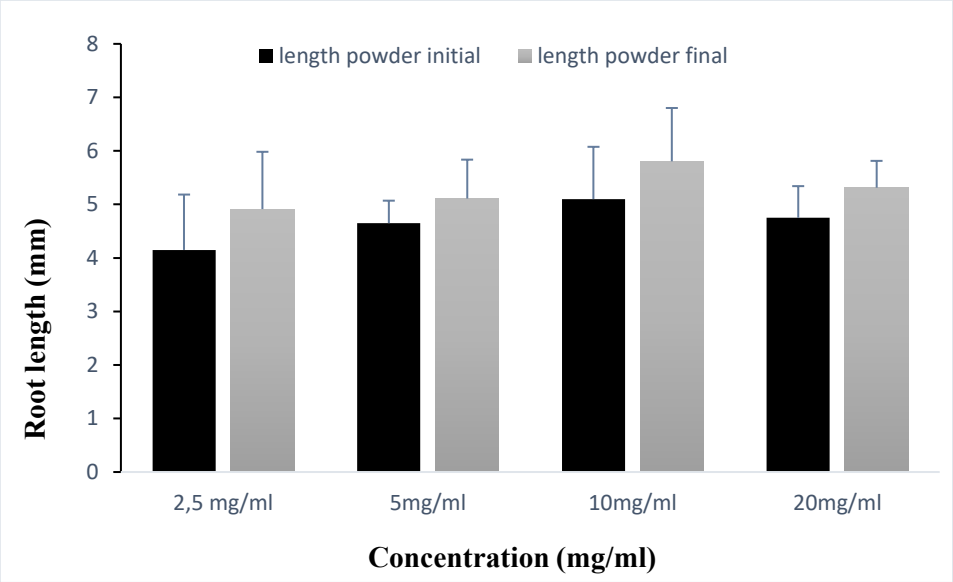

Figure 41. Roots length before and after treatment with plant powder of *E. alata.*

Pre-treatment, the longest roots occurred at 10 mg/mL while the shortest were at 2.5mg/mL; post-treatment, 10 mg/mL stayed the longest root and 2.5 mg/mL remained the minimum length despite growth. All concentrations exhibited increased root length. the strongest progression was at 10 mg/mL (optimal response) (Figure.41).

3.2.Cell phases

A. Prophase

- *Aristolochia longa:*

Analysis of data showed that the prophase cells number was significantly higher among all treatments. It appears that *A. longa* extracts did not affect the prophase cells number (Figure.46)

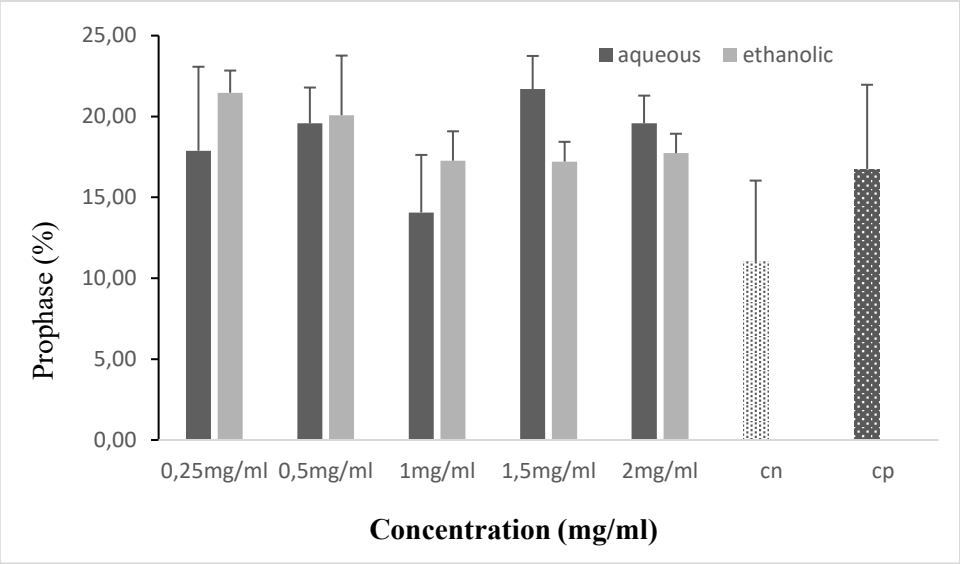

Figure 46. The effect of *A. longa* extracts on prophase in the root cells of *A.cepa*

The highest prophase cells number was marked in aqueous extract under the treatment of 1.5 mg/mL (21.69±2.05%). While the lowest cells number was obtained under the treatment with 1 mg/mL (14.06±3.56%). On the other hand, the highest prophase cells number was observed in the ethanolic treatment with 0.25 mg/mL (21.47±1.37%) of the ethanolic extract while the lowest cells umber was observed at 1.5 mg/mL (17.21±1.82%).

Powder of *Aristolochia longa*:

The data analysis showed that the powder decoction had no significant effect on the frequency of cells in prophase, as compared to the control group (Figure.47)

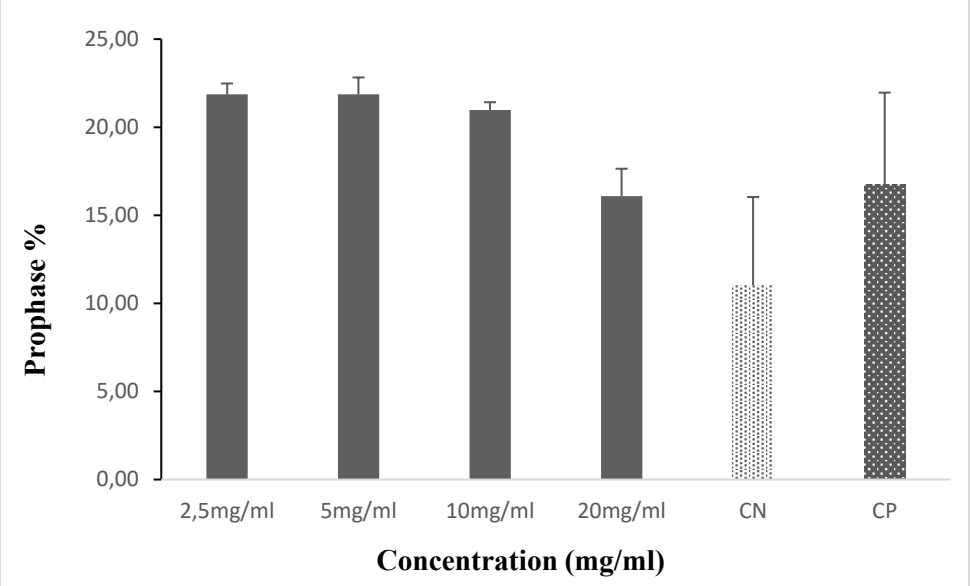

Figure 47. The effect of *A. longa* decoction powder on prophase in the root cells of *A. cepa.*

The positive control (CP) showed a prophase of (16.74± 5.22%), compared to (11.04± 5.00%) for the negative control (CN).

The powder decoction induced the highest prophase at 5 mg/ml (21.87 ± 0.95%) and the lowest value at 20 mg/ml (16.09 ± 1.55%).

- Ephedra alata:

The graph displays a non-linear pattern, a noticeable slight decrease is observed at the intermediate concentrations aqueous and ethanolic, other concentrations looks like it hadn't an effect on other prophase cells (Figure.48).

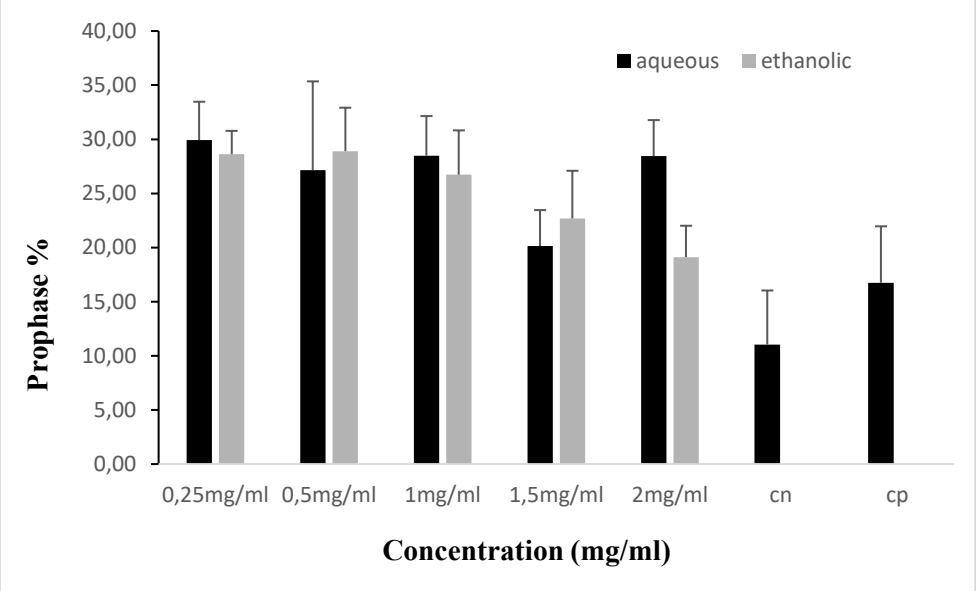

Figure 48. The effect of *E. alata* extracts on prophase in the root cells of *A.cepa.*

The positive control (CP) showed a prophase of (16.74 ± 5.22%), compared to (11.04± 5.00%) for the negative control (CN).

The aqueous extract induced the highest prophase at 0.25 mg/ml (29.93± 3.54%) and the lowest value at 1.5 mg/ml (20.14 ± 3.33%). For the ethanolic extract, the highest prophase activity was observed at 0.5 mg/ml (28.91± 4.01%), while the lowest was obtained at 2 mg/ml (19.11 ± 2.89%).

Powder of *Ephedra alata*:

Analysis data demonstrated a significant decrease in prophase effected by decoction powder of *Ephedra alata* in higher concentrations (Figure.49).

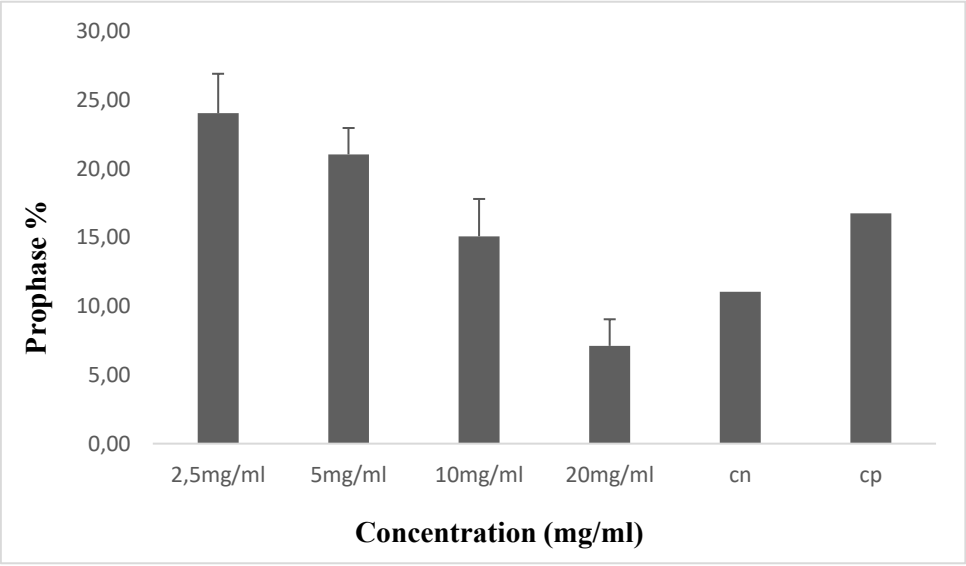

Figure 49. The effect of *E. alata* decoction powder on prophase in the root cells of *A.cepa*

The positive control (C^+) showed a prophase of (16.74 ± 5.22%), compared to (11.04± 5.00%) for the negative control (C^-).

The powder decoction induced the highest prophase at 2.5 mg/ml (24.02 ± 2.87%) and the lowest value at 20 mg/ml (7.11 ± 1.93%).

B. Telophase

- *Aristolochia longa*:

As the concentration *A. longa* extracts increased, a gradual decline in the number of root cells of *A. cepa* was observed (Figure.58).

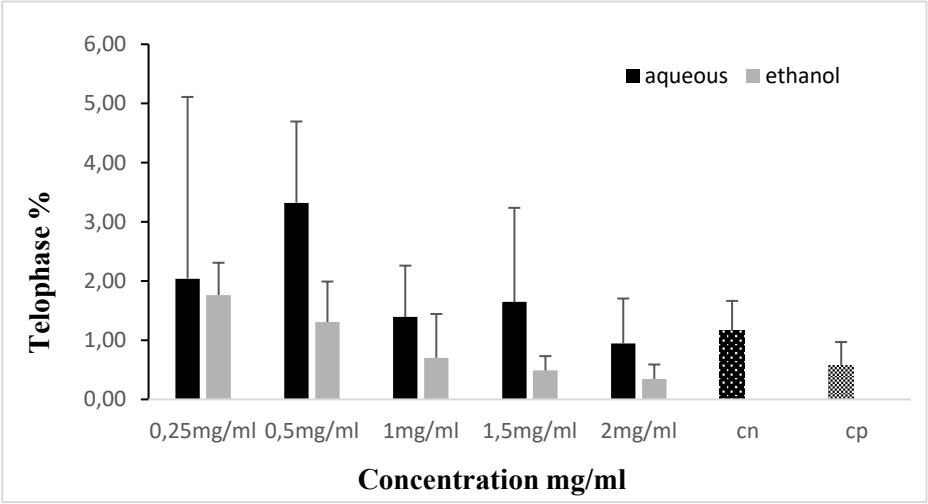

Figure 58. The effect of *A. longa* extracts on Telophase in the root cells of *A.cepa.*

The positive control (CP) showed a telophase of (0.56± 0.40%), compared to (1.16 ± 0.50%) for the negative control (CN).

The aqueous extract induced the highest telophase at 0.25 mg/ml (3.32 ± 1.37%) and the lowest value at 2 mg/ml (0.94 ± 0.76%). For the ethanolic extract, the highest telophase activity was observed at 0.25 mg/ml (1.76± 0.55%), while the lowest was obtained at 2 mg/ml (0.35 ± 0.24%).

Powder of *Aristolochia longa*:

The data analysis showed a high effect of decoction powder on *A. cepa* root cells, when concentrations increase telophases decreases (Figure.59).

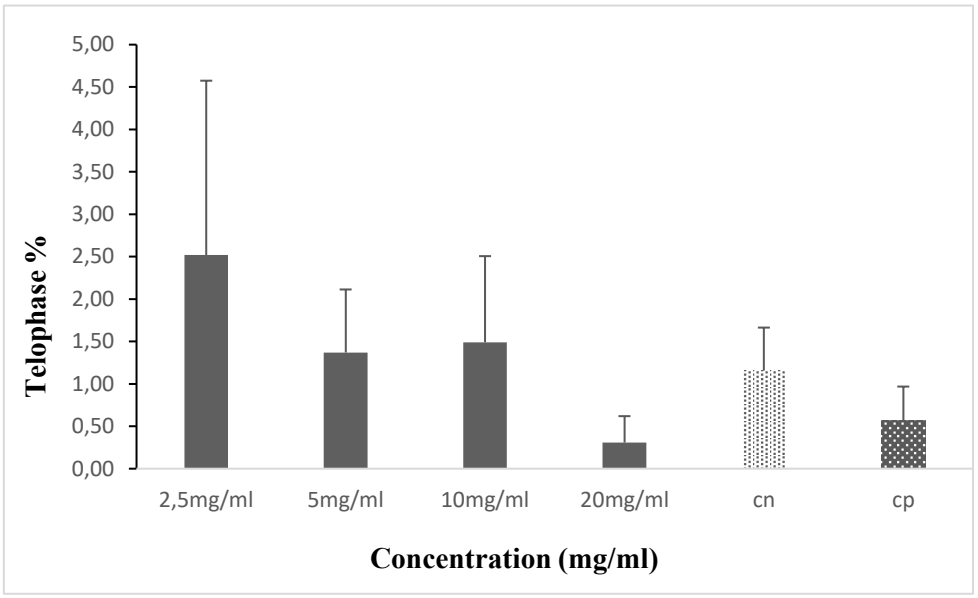

Figure 59. The effect of *A. longa* decoction powder on Telophase in the root cells of *A.cepa.*

The positive control (CP) showed a telophase of (0.56 ± 0.40%), compared to (1.16 ± 0.50%) for the negative control (CN).

The powder decoction induced the highest telophase at 2.5 mg/ml (2.52 ± 2.05%) and the lowest value at 20 mg/ml (0.31 ± 0.31%).

- *Ephedra alata:*

Analysis data marked nearly an absence of Telophase in the highest concentrations of aqueous extract in addition of sharp decrease in telophase cells which means a higher effect of *E. alata* extracts on *A. cepa* root cells (Figure.60).

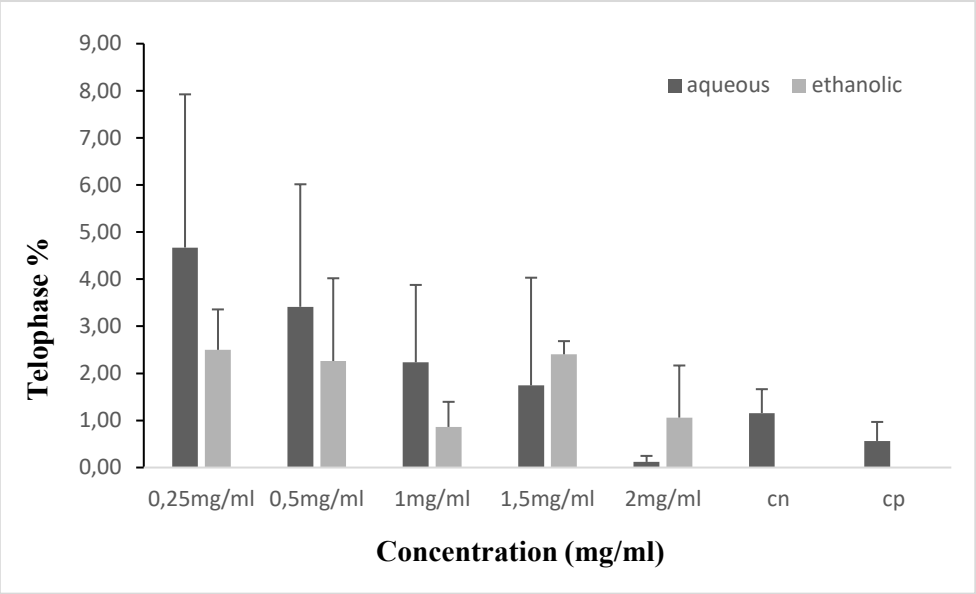

Figure 60. The effect of *E. alata* extracts on Telophase in the root cells of *A.cepa.*

The positive control (C^+) showed a telophase of (0.56 ± 0.40%), compared to (1.16 ± 0.50%) for the negative control (C^-).

The aqueous extract induced the highest telophase at 0.25 mg/ml (4.67± 3.25%) and the lowest value at 2 mg/ml (0.12 ± 0.12%). For the ethanolic extract, the highest telophase activity was observed at 0.25 mg/ml (2.50 ± 0.58%), while the lowest was obtained at 1 mg/ml (0.86 ± 1.10%).

Powder of *Ephedra alata:*

Analysis data demonstrated a significant decrease in Telophase effected by decoction powder of *Ephedra alata* in higher concentrations (Figure.61).

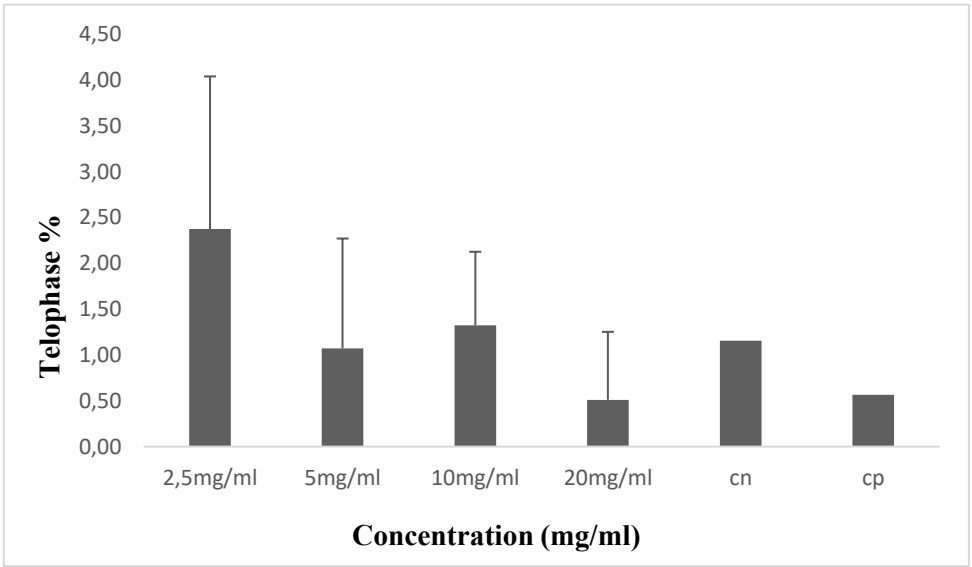

Figure 61. The effect of *E. alata* decoction powder on Telophase in the root cells of *A. cepa.*

The positive control (C^+) showed a telophase of (0.56 ± 0.40%), compared to (1.16 ± 0.50%) for the negative control (C^-).

The powder decoction induced the highest telophase at 2.5 mg/ml (2.37 ± 1.66%) and the lowest value at 20 mg/ml (0.51 ± 0.74%).

4. Evaluation of genotoxicity

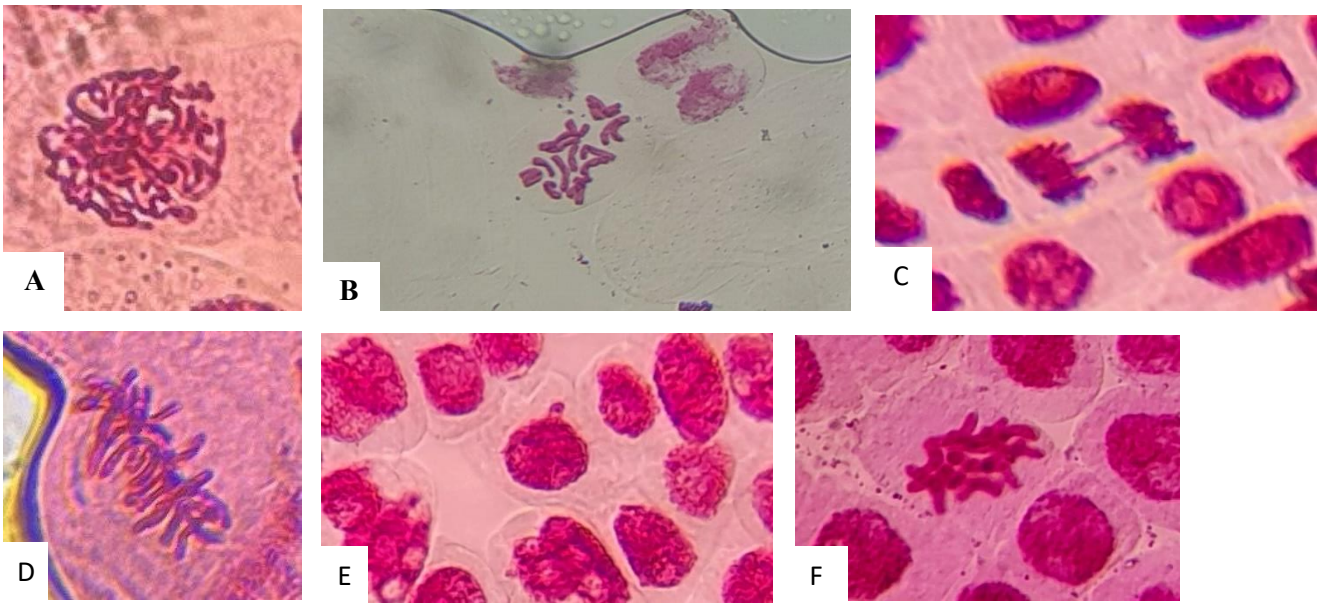

Figure 62. Chromosomal aberrations induced by *A. longa* and *E. alata* extracts and decoction. A: polyploid in prophase; B & F: C- metaphase; C: Chromosomal bridge on anaphase. D: polyploid in metaphase; E: nuclear budding.

Discussion

Worldwide, cancer remains a leading cause of morbidity and mortality, with nearly **2**0 million new cases and 10 million deaths reported in 2020, breast, lung, colorectal, prostate, and stomach cancers being most common (Sung et al., 2021). In Algeria, many patients turn to traditional herbal remedies, yet their use is often anarchic, uncontrolled, and unregulated, reflecting a widespread lack of knowledge of safe dosages among both patients and healthcare providers (Helali et al., 2022). Such practices can lead to toxicity, adverse interactions, or reduced therapeutic efficacy, especially in serious diseases.

Therefore, this study has a purpose of evaluating the ethnopharmacological uses, phytochemical properties, and cytogenotoxicity of two widely used Algerian medicinal plants *Aristolochia longa L*. and *Ephedra alata Decne* using the *Allium cepa* assay.

Our main results demonstrated that both of *Aristolochia longa* and *Ephedra alata* are mainly used for treating different kind of cancer and prostate, with citations of 130 to 140 respondents, our study showed us also that the ethanolic extract in *Aristolochia longa* and *Ephedra alata* achieved the highest recovery (17.23%) and (14.76%) respectively more than aqueous extract and these two plants have highly properties of polyphenolic between 886.85± 14.83 mg GAE/g and 1014.07± 54.07 mg GAE/g for ethanolic extract and decoction powder for *E. alata,* and between 354.81± 70.01 mg GAE/g and 832.59± 15.01 mg GAE/g for ethanolic extract and decoction powder for *A. longa*, flavonoids total content were between 500.83±68.05 mg QE/g and 1165.28± 45.99 mg QE/g on 20 mg/ml treatment in decoction powder, tannins total content were 75.56 mg TA/g and 114.07 mg TA/g in the treatment with 20 mg/ml, besides that, the results of allium cepa essay demonstrate that both of the plant have a higher effect an allium cepa mitotic cells especially *Aristolochia longa* with high toxicity in higher concentrations with the appearance of different aberrations and the highest aberration appeared for both of the plant was polyploidy.

In Algeria, many patients with different kind of diseases having lack of knowledge in using medicinal plant with appropriate dosage.

therefore, an ethnopharmacology study has been covered different region in west of Algeria (Tiaret, Sougeur, Oued Souf, Oran, mehdia, El Bayadh, Bechar, Media), the result demonstrated that among the 200 respondents, most of whom were middle-aged, cancer was cited 135-140 times as the highest treatment of *Aristolochia longa*. Prostate disorders followed with 25–28 mentions. All other therapeutic applications were reported fewer than five times each. The respondents also mentioned that *Aristolochia longa* has a great danger if

the user surpasses its limited dose which was according to them nearly 100g/1kg of honey, and 20 to 25g/1L in case of using decoction.

Other study by Benarba et al., (2014) on *Aristolochia longa* valuated its effects on bone resorption markers in postmenopausal Algerian women with breast cancer. Patients were divided into three groups: *A. longa* users (n = 54), non-users (n = 24), and a control group (n = 32). Bone resorption markers (PYD and DPD) and kidney function indicators (creatinine, uric acid, urea) were measured. The intake of 1 g of *A. longa* led to a significant increase in both kidney markers and bone resorption markers. These results indicate that *A. longa* intake harms kidney function and promotes bone loss, it has not been other scientific approved studies which support that *Aristolochia longa* treat cancer or limited its symptoms, so Benarba et al., (2014) study confirms that *Aristolochia longa* has a danger effect on the users and patients, probably because it contains aristolochic acids (AAs), which are proven to be both nephrotoxic (damaging to kidneys) and carcinogenic (cause cancer) (Benarba *et al* ,2014), The difference comes from people trusting tradition without proof, while science needs strong evidence. *A. longa* seems helpful to some, but studies show its toxic effects.

The same respondents were asked about the therapeutic uses of *Ephedra alata*, Cancer treatment overwhelmingly dominates the reported therapeutic uses of *E. alata*, with approximately 118–120 citations, making it the most frequently cited indication. Weight loss follows as a distant second, with about 25–27 mentions, while prostate problems come next with 15–17 citations. Other conditions such as respiratory issues, microbial infections, fertility problems, digestive disorders, cardiovascular diseases, and Anemia are rarely mentioned, each receiving fewer than 5 citations. These results indicate that cancer is the primary reported use of *E. alata*, with weight loss and prostate health as secondary, and all other applications remaining marginal with respecting the appropriate dosage which was according to the respondents about 5 sticks of *E. alata* in a glass or decoction of 250g/ 7L of water.

The study of Mohammed et al., (2023) aimed to investigate the alkaloid content and biological activity of the local medicinal plant *Ephedra alata*, focusing on its stems. Alkaloid-rich and crude extracts were prepared using optimized extraction methods. Identification and quantification of alkaloids, particularly ephedrine, were performed using TLC and HPLC techniques, revealing concentrations of 8 ppm in the alkaloid-rich extract and 5 ppm in the crude extract. The cytotoxic effects of the extracts were tested on human breast cancer cell

lines (MDA-MB-231) and normal human lymphocytes (HLs). The results showed that *E. alata* demonstrate a significant anti-cancer activity. The crude extract induced 50.11% cell death at 75 mg/ml after 72 hours, and 50.63% inhibition at 15 mg/ml after 24 hours (Mohammed et al, 2023).

These results confirms that *E. alata* shows promising anti-cancer potential, when used at appropriate concentrations. These findings support its possible application in cancer therapy, warranting further investigation.

Ethnopharmacology's exploration of traditional plant remedies has driven phytochemical research into polyphenols over 8,000 plant derived compounds noted for antioxidant and anti-inflammatory effects that may help regulate metabolism and prevent chronic diseases, though isolated intake can pose risks (Cory et al., 2018). Key subclasses include flavonoids, which contribute to pigmentation and ecological functions in flowers, fruits, and roots (Santos et al., 2017), and tannins, high molecular weight polyphenols in cereals, legumes, and fruits that protect plants and offer antimicrobial and cardioprotective benefits (Smeriglio et al., 2016).

Therefore, building on our ethnopharmacological work, our study then proceeded to phytochemical analysis.

Alcohols, as polar compounds, occupy an intermediate position between lipophilicity and hydrophilicity (Wade, 2024). Moreover, at concentrations above 20%, alcohol solutions are self-preserving and this require less maintenance than water-based systems (Abubakar et Haque, 2020).

Extraction yields differed by solvent, the ethanolic extract in *Aristolochia longa* and *Ephedra* alata achieved the highest recovery (17.23%) and (14.76%) respectively, highlighting ethanol's effectiveness in extracting a broad spectrum of phytochemicals.

In *A. longa*, the phenolic content increased according to the rise of the extract concentrations. Study of Beldi et al.,(2022), The total phenol content of *A. longa* extracts showed that the plant was found to have high levels of phenolic compounds (values between 46.33 ± 8.39 176.56 ± 4.00 mg/g. Total phenolic content of our study was about 354.81± 70.01 mg GAE/g, in the treatment with 2 mg/mL of ethanolic extract and a higher value of 832.59± 15.01 mg GAE/g, in the treatment with 20 mg/mL in decoction powder, These differences with previously published studies may stem from differences in geographic

origin, plant parts used, harvest timing, soil composition, storage conditions, and choice of extraction solvent. Variations in extract concentrations and the amount of plant powder employed could also contribute.

For *E. alata*, the phenolic content increased according to the rise of the extract concentrations also, study made by Chebouat et al., (2023) on *E. alata* plant with the following protocol: Add 1 ml of Folin-Ciocalteu's reagent diluted 10 times in distilled water; Then leave to act for 5 minutes before adding 0.8 mL of 7.5% sodium carbonate. After 30 min of incubation at room temperature and protection from light, read the absorbance of a UV-vis spectrophotometer at 760 nm, the results demonstrated that total phenolic content was 46,66 ± 3,06 mg GAE/g, our results marked much higher total phenolic content with value of 886.85± 14.83 mg GAE/g and a higher value of 1014.07± 54.07 mg GAE/g, in the treatment with 20 mg/mL in decoction powder, Variations from earlier studies might be explained by differing geographic origins, plant parts, harvest dates, soil composition, storage methods, and extraction solvents. Additionally, disparities in experimental conditions such as extract concentrations and the amount of plant material used may also play a significant role.

The flavonoid and Tannins content increased in response to the rise of the extract concentrations in *A. longa*, El Idrissi et al., (2021) study demonstrated the total flavonoids content with 54.21 mg QE (Quercetin equivalent)/g, Attou et al., (2022) realized a study which resulted the maximum tannin content value was noted in roots N-butanol fraction (3.90±0.24 mg QUE/g DM) followed by leaves ethyl acetate fraction (3.03±0.01 mg QUE/g DM), our results in total flavonoids and tannins content were 500.83±68.05 mg QE/g 1165.28± 45.99 mg QE/g on 20 mg/ml treatment in decoction powder and 75.56 mg TA/g and 114.07 mg TA/g in the treatment with 20 mg/ml respectively. The results are clear that there is a huge difference in the results, Variations from earlier studies might be also explained by differing geographic origins, plant parts, harvest dates, soil composition, storage methods, and extraction solvents. Additionally, disparities in experimental conditions such as extract concentrations and the amount of plant material used may also play a significant role.

The flavonoid and tannins content increased in response to the rise of the extract concentrations in *E. alata*, according to khaldi et al., (2023) study results, 133.25±0.11 mg QE/g for total flavonoids content and 20.76±0.19 mg CE/g for total tannins content was obtained as data, our study results shows that total flavonoids content reach 548.89±40.83 mg QE/g in the treatment with 2 mg/mL of the aqueous extract and 1501.39± 15.01 mg QE/g in the treatment with 20 mg/mL for decoction powder, total tannins content 123.33±7.29 mg

TA/g in the ethanolic extract at 2 mg/ml and tannin content peaks at approximately 265 mg TA/g with treatment of 20 mg/ml in decoction powder. The results clearly show significant differences compared to earlier studies, which may be attributed to variations in plant characteristics (geographic origins, specific plant parts used, harvest dates, and soil composition), preparation methods (storage conditions and extraction solvents), and experimental parameters (extract concentrations and quantity of plant material utilized.

According to Wada et Matsumoto, K. (2009) and Takumi et al., (2008) studies about phenolic content and flavonoids, phytochemical compounds, while celebrated for their health benefits, it could exhibit significant genotoxic potential that can lead to chromosomal lesions, therefore, our study proceeded to evaluate the cytogenotoxicity of *Aristolochia longa* and *ephedra alata.*

Our study for *A. longa* demonstrates that regarding the aqueous and ethanolic extracts, there is an increase in the length of roots is noted at all concentrations, the increase in ethanolic extract was higher than aqueous extract in most concentrations, in decoction powder all concentrations exhibited increased root lengths except for 10 mg/ml showed a decrease in length by 0.5mm.

In mitotic index, Analysis of data demonstrated that *A. longa* extracts noticeably affect the mitotic index (MI %) and mitotic phases of *A. cepa* meristematic root cells. The rate of mitotic index decreased when the concentrations of the extracts increased from 34% to 22% in the following concentrations (0.25 – 0.5 – 1 – 1.5 – 2 mg /ml), same analysis in decoction powder marked a decrease from 32% to 20% in following concentrations (2.5, 5, 10 and 20 mg/ml)

Analysis showed that the prophase cells number was significantly higher among all treatments in phase index which means *A. longa* did not affect the prophase cells number. Other index phases such as (metaphase, anaphase, telophase) showed that the plant extracts of *A. Longa* had significantly an effect on *A. cepa* meristematic root cells specially in higher concentrations (1.5 and 2mg/ml) and for decoction powder (10 and 20 mg/ml). The aberrations index suggests a moderate to high effect on *A. cepa* root cells, with a higher level of aberrations in aqueous extract than ethanolic one reaching 4% in approximal 5000 counted cells in each concentration for each phase index. Polyploidy aberration was the highest aberrations repeated in genotoxicity test with the mean aberration frequency across the five

concentrations with 68.2 in aqueous extract, 64.2 in ethanolic extract, 85.5 in decoction powder which results that the powder has much effect an *A. cepa* root cells.

Study made by Ventura et al., (2021) demonstrate that traditional medicinal plants *Aristolochia labiata* and *A. triangularis* contain carcinogenic aristolochic acids (AAs). When tested on onion (*Allium cepa*), decoctions of these plants (4-32 g/L) showed severe toxicity, concentrations ≥4 g/L reduced seed germination (32 g/L prevented it entirely), slowed seedling growth, and inhibited cell division (reduced mitotic index). Higher concentrations (8-16 g/L) disrupted cell cycles by trapping nuclei in the G0/G1 phase and caused abnormal heteropycnotic nuclei formation. Phytochemical analysis confirmed abundant alkaloids including toxic AAs as the likely cause. Despite there is no study showing the toxic effect of *Aristolochia longa*, this study with the same gender of *Aristolochia longa* confirms the toxicity of this plant and its danger especially in powder decoction, this toxicity could be explained by the Aristolochic acids known for its DNA damage and provoke cancer risks in humans, never then less, the high content of total phenolic and flavonoids, this study strongly warns against consuming these decoctions despite their traditional use.

Same study was conducted for *E. alata*, our results showed regarding the aqueous and ethanolic extracts, ethanolic extract exceeded the aqueous extract root length with slight difference, the same for decoction powder.

In mitotic index, Analysis of data in *E. alata* demonstrated a noticeable decrease in mitotic cells in higher concentrations in extracts ethanolic and aqueous extracts (MI, %) and mitotic phases of A. cepa meristematic root cells. The rate of mitotic index decreased when the concentrations of the extracts increased from 54% to 32% in the following concentrations (0.25 – 0.5 – 1 – 1.5 – 2 mg /ml), same analysis in decoction powder marked a decrease from 35% to nearly 10% in following concentrations (2.5, 5, 10 and 20 mg/ml).

Analysis showed a noticeable slight decrease the intermediate concentrations (1 mg/ml) in both aqueous and ethanolic extract, other concentrations looks like it hadn’t an effect on prophase cells. Other index phases such as (metaphase, anaphase, telophase) showed that the plant extracts of *E. alata* had significantly high effect on *A. cepa* meristematic root cells specially in higher concentrations (1.5 and 2mg/ml) and for decoction powder (20 mg/ml). The aberrations index suggests a moderate to high effect on *A. cepa* root cells specially in 0.5 and 1.5 mg/ml in aqueous and ethanolic extract and 5 mg/ml for decoction powder, with a higher level of aberrations in aqueous extract than ethanolic one reaching 4% in approximal

5000 counted cells in each concentration for each phase index. Polyploidy aberration was the highest aberrations repeated in the aberrations index with the mean aberration frequency across the five concentrations with 49 in aqueous extract, 43.6 in ethanolic extract, 37 in decoction powder which results that the powder has much effect an *A. cepa* root cells.

Study made by Mohammed et al., (2023), *Ephedra alata* extracts (alkaloid-rich and crude aqueous) and pure ephedrine were tested on *Allium cepa* root tips for 5 hours at five concentrations. Both extracts exhibited an IC_{50} of 35 mg/mL, indicating sublethal effects on cell viability. At 75 mg/mL, a high frequency of chromosomal abnormalities was observed – primarily chromosome stickiness attributed to disrupted nucleic acid metabolism and a eugenic activity. These chromosomal and nuclear aberrations, resulting from the extracts' chemical composition, suggest the plant's potential as an antitumor agent. The two results conclude that *Ephedra alata* has a potential of toxicity in high concentrations, it could be because of the high content of total phenolic and flavonoids, but it also confirms it capacity and potential for blocking cell division which means it can block Cancer cells.

Conclusion & perspectives

Our ethnopharmacological survey in western Algeria confirms that *Aristolochia longa* and *Ephedra alata* are widely used to treat cancer, prostate disorders, and other metabolic ailments despite the absence of formal regulation and clear dosing guidelines, which can expose users to toxicity and harmful interactions.

Phytochemical analysis shows that ethanol extracts concentrate substantially more polyphenols, flavonoids, and tannins than aqueous preparations, highlighting their strong antioxidant and anti-inflammatory potential.

In vitro *Allium cepa* assays demonstrate a dose-dependent inhibition of mitosis: *A. longa* produces the most pronounced suppression of cell division with frequent chromosomal abnormalities particularly polyploidy while *E. alata* induces moderate cytotoxic effects alongside signs of antitumor activity. The observed renal and bone toxicity linked to *A. longa* further underscores the risks associated with its unregulated traditional use. These findings point to an urgent need to develop and implement standardized protocols that define safe dosages, optimized formulations, and validated extraction methods to conduct comprehensive pharmacokinetic and toxicological evaluations; to pursue mechanistic studies elucidating molecular modes of action and to undertake controlled clinical trials that compare these botanical remedies against established therapies. Such an integrated approach melding rigorous scientific validation with respect for traditional knowledge will be essential to transform these local plants into reliable, effective, and safe treatment options while safeguarding both patient health and biodiversity.

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