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Presented by:

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Title

***Alpinia galanga*: Ethnopharmacology, phytochemistry,
cytotoxicity and genotoxicity.**

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Mémoire

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Intitulé

***Alpinia galanga* : Ethnopharmacologie, phytochimie, cytotoxicité
et génotoxicité**

Soutenu publiquement le :

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

الملخص

Alpinia galanga هي نبتة طبية تُستخدم على نطاق واسع في الطب التقليدي الجزائري لعلاج اضطرابات الجهاز التنفسي والهضمي وأمراض النساء. هدفت هذه الدراسة إلى التحقيق في تطبيقاتها الإثنوفارماكولوجية، وتركيبها الكيميائي النباتي في المستخلصات المائية، و الهيدروإيثانولي، والمحضرة عن طريق النقع، إضافة إلى تقييم تأثيراتها السمية الخلوية والجينية باستخدام اختبار *Allium cepa*

أكد الاستطلاع الإثنوفارماكولوجي، الذي أجري في 16 ولاية جزائرية بمشاركة 200 شخص، الاستخدام المتكرر لـ *A. galanga*، خاصة في شكل مغلي أو منقوع، وكانت الأمراض التنفسية الأكثر ذكراً من حيث دواعي الاستعمال.

أظهرت التحاليل الكيميائية النباتية أن المستخلص الهيدروإيثانولي يحتوي على نسب أعلى من البوليفينولات والفلافونويدات والتانينات مقارنة بالمستخلص المائي، مما يدل على كفاءة الإيثانول كمذيب. ومن المثير للاهتمام أن المنقوع أظهر أيضاً وجوداً ملحوظاً لهذه المركبات الفعالة، خاصة في التركيزات العالية، مما يؤكد أهميته كطريقة تقليدية فعالة للاستخلاص.

في اختبارات السمية الخلوية، أظهرت المستخلصات المائية والإيثانولية تأثيرات تعتمد على الجرعة على عدد وطول الجذور، مع تحفيز عند الجرعات المنخفضة وتثبيط عند الجرعات العالية. أما المنقوع فقد أظهر تحفيزاً معتدلاً لانقسام الخلايا، مع أعلى مؤشر انقسام عند تركيز 10 ملغ/مل

أشارت نتائج تحليل مراحل الانقسام الخيطي إلى سيطرة الطور التمهيدي في جميع المعالجات، في حين انخفضت تكرارات الطور الاستوائي والنهائي بشكل ملحوظ عند التركيزات العالية، خاصة في المستخلص الهيدروإيثانولي حيث اختفت الخلايا في الطور الاستوائي تماماً عند تركيز 1-2 ملغ/مل.

أظهر تقييم السمية الجينية عدة تشوهات كروموسومية، منها تعدد الصبغيات، والانقسام الاستوائي غير الطبيعي، والتلاصق، والكروموسومات المتأخرة، وكانت أعلى نسبة من التشوهات عند الجرعات المنخفضة (0.25 ملغ/مل). ولحسن الحظ، أظهر المنقوع أقل سمية جينية.

تشير هذه النتائج إلى أن *A. galanga* تحتوي على مركبات نباتية فعالة ذات تأثير سام للخلايا، خاصة عند التركيزات العالية. ورغم أن الاستخدام التقليدي يدعم أهميتها العلاجية، إلا أن تأثيراتها السمية الخلوية والجينية تتطلب الحذر وإجراء المزيد من الدراسات لتحديد الجرعات الآمنة.

علاوة على ذلك، يجب دراسة هذا التأثير السام للخلايا بشكل أعمق والاستفادة منه لإبراز خصائصه المحتملة كمضاد للسرطان، مما قد يساهم في تطوير علاجات نباتية مضادة للسرطان.

الكلمات الدالة

Alpinia galanga، علم الأدوية العرقية، الكيمياء النباتية، التسمم الخلوي و الجيني، الطب التقليدي، الجزائر.

Abstract

Alpinia galanga is a medicinal plant widely used in traditional Algerian medicine for treating respiratory, digestive, and gynecological disorders. The present study aimed to investigate its ethnopharmacological applications, phytochemical composition of aqueous, ethanolic, and infusion preparations of *A. galanga* rhizomes, and their cytotoxic and genotoxic effects through the *Allium cepa* bioassay.

The ethnopharmacological survey, conducted across 16 Algerian regions with 200 participants, confirmed the frequent uses of *A. galanga* primarily in decoction and infusion forms, with respiratory conditions being the most cited indications. Phytochemical evaluation revealed that the hydroethanolic extract exhibited higher contents of polyphenols, flavonoids, and tannins compared to the aqueous extract, demonstrating the superior solvent efficiency of ethanol. Interestingly, the infusion also showed a considerable presence of these bioactive compounds, especially at higher concentrations, highlighting its relevance as a traditional and effective method of extraction.

In the cytotoxicity assays, both aqueous and hydroethanolic extracts showed dose-dependent effects on root number and length, with stimulation at lower doses and inhibition at higher concentrations. The infusion, however, showed moderate stimulation of cell division, with a peak mitotic index at 10 mg/mL (18.68%).

Analysis of mitotic phases indicated prophase dominance across all treatments, while metaphase and telophase frequencies decreased significantly at higher concentrations, particularly in hydroethanolic extract where metaphase cells disappeared completely at 1–2 mg/mL. Genotoxicity assessment showed a variety of chromosomal aberrations, including polyploidy, C-metaphase, stickiness, and lagging chromosomes, with the highest frequency of anomalies at lower doses (0.25 mg/mL). Fortunately, the infusion showed the lowest genotoxic potential.

These results suggest that *A. galanga* possesses active phytochemical compounds with cytotoxic potential, especially at high concentrations. While traditional use supports its therapeutic relevance, its cytogenotoxic effects warrant cautious application and further studies to define safe dosage thresholds. Moreover, this cytotoxic potential should be further investigated and valorized for its promising anticancer properties, as it may contribute to the development of plant-based anticancer therapies.

Keywords: *Alpinia galanga*; Ethnopharmacology; Phytochemistry; Cytogenotoxicity; Traditional medicine; Algeria.

Résumé

Alpinia galanga est une plante médicinale largement utilisée dans la médecine traditionnelle algérienne pour traiter les troubles respiratoires, digestifs et gynécologiques. La présente étude visait à explorer ses applications ethnopharmacologiques, la composition phytochimique des préparations aqueuses, hydroéthanoliques et en infusion des rhizomes d'*A. galanga*, ainsi que leurs effets cytotoxiques et génotoxiques à travers le test bio *Allium cepa*.

L'enquête ethnopharmacologique, menée dans 16 régions algériennes auprès de 200 participants, a confirmé l'usage fréquent d'*A. galanga*, principalement sous forme de décoction et d'infusion, les affections respiratoires étant les indications les plus citées. L'évaluation phytochimique a révélé que l'extrait hydroéthanolique présentait une teneur plus élevée en polyphénols, flavonoïdes et tanins que l'extrait aqueux, démontrant ainsi la meilleure efficacité du solvant éthanolique. Fait intéressant, l'infusion a également montré une présence considérable de ces composés bioactifs, en particulier à fortes concentrations, soulignant sa pertinence comme méthode traditionnelle et efficace d'extraction.

Dans les tests de cytotoxicité, les extraits aqueux et hydroéthanoliques ont montré des effets dose-dépendants sur le nombre et la longueur des racines, avec une stimulation à faibles doses et une inhibition à fortes concentrations. L'infusion a quant à elle montré une stimulation modérée de la division cellulaire, avec un indice mitotique maximal à 10 mg/mL (18,68 %). L'analyse des phases mitotiques a indiqué une dominance de la prophase dans tous les traitements, tandis que les fréquences de la métaphase et de la télophase ont diminué significativement à fortes concentrations, en particulier dans l'extrait hydroéthanolique où les cellules en métaphase ont complètement disparu à 1–2 mg/mL. L'évaluation de la génotoxicité a montré diverses aberrations chromosomiques, notamment la polyploïdie, la c-métaphase, l'adhérence chromosomique et les chromosomes en retard, avec une fréquence maximale d'anomalies à faibles doses (0,25 mg/mL). Heureusement, l'infusion a révélé le plus faible potentiel génotoxique.

Ces résultats suggèrent qu'*A. galanga* possède des composés phytochimiques actifs ayant un potentiel cytotoxique, surtout à fortes concentrations. Bien que son usage traditionnel soutienne sa pertinence thérapeutique, ses effets cytogénotoxiques nécessitent une application prudente et des études supplémentaires afin de définir des seuils de dosage sûrs. Par ailleurs, ce potentiel cytotoxique devrait être davantage étudié et valorisé pour ses propriétés anticancéreuses prometteuses, pouvant contribuer au développement de thérapies anticancéreuses à base de plantes.

Mots clés : *Alpinia galanga* ; Ethnopharmacologie ; Phytochimie ; Médecine traditionnelle ; Algérie.

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Dedication

To my precious mother,

This work is first and foremost dedicated to you... the woman whose love knows no limits, whose sacrifices are countless, and whose prayers have carried me through the most difficult moments.

Your strength has been my foundation, your patience my lesson, and your belief in me my driving force. In silence, you gave everything, asking for nothing in return. Every page of this work holds a part of you. In the sleepless nights you endured for me, in the words of encouragement you whispered, and in the hope, you never let me lose.

You are my first teacher, my strongest support, and my forever inspiration. This accomplishment is not mine alone. It is ours.

To my dear father,

Thank you for your wisdom, your quiet strength, and your constant support. Your presence has been a comfort and a guide.

To my beloved sisters

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You are my forever friends, my daily joy, and my greatest cheerleaders. I am endlessly grateful for the bond we share and the love that connects us.

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This thesis is for all of you, with deep love and eternal gratitude.

Inas Rodina

Introduction

Introduction

Herbal medicine has long been used to treat illnesses and maintain health, especially in low-income communities where it remains a vital, affordable solution passed down through generations (Aït Abderrahim et al., 2019). Given the high cost and limitations of modern treatments, many people increasingly turn to medicinal plants as a trusted, affordable alternative (Taïbi et al., 2020). Currently, there is a growing movement to combine traditional healing methods with modern medicine, creating a more holistic and connected approach to health (Coumaré 2021).

Medicinal plants are indeed a valuable source of active compounds with diverse biological effects, commonly used to treat and manage a wide array of diseases (Benarba 2016). People tend to view medicinal plants as a natural and safer choice since they usually cause fewer side effects compared to modern synthetic drugs (Anywar et al., 2021). However, Sponchiado et al. (2015) point out that the assumption of complete safety, stemming from centuries of traditional uses, can be misleading. Not all plants have been properly studied and tested for toxicity, and some may carry hidden risks and could actually harm our DNA or health.

Algeria's rich and varied history, shaped by various civilizations, combined with its rich biodiversity has created a rich ethnic and cultural mosaic replete with an abundant wealth of traditional healing practices. These traditional practices offer not only valuable knowledge but also a unique opportunity for scientific exploration (Taïbi et al., 2020).

Alpinia galanga Willd. (Zingiberaceae), commonly known as galangal, and in Algeria as Khorjlan, is an important cultivated medicinal crop in India (Shukla et al., 2017) and is also widely used and consumed in Algeria. Despite its longstanding popularity, no comprehensive study has to date investigated its various traditional uses and applications, nor investigated its potential benefits and toxicity. This valuable medicinal plant is known abroad for its broad range of therapeutic effects and contains numerous bioactive compounds that underpin its diverse pharmacological properties (Verma, 2011).

In this context, the aim of the current research is threefold: (i) to systematically document and preserve Algerian's cultural knowledge regarding the traditional uses of *Alpinia galanga*, (ii) to evaluate the phytochemical composition of various extracts of this species, and (iii) to carry out a scientific investigation of the safety profile through assessing its cytotoxic and genotoxic potentials.

Hence, this study will document and safeguard the main natural products used in Algeria, along with their modes of preparation and administration, to assess traditional knowledge of their beneficial uses and the risks associated with misuse. Ultimately, this research aims to contribute to the development of a national strategy for standardizing natural product use, ensuring optimum quality, efficacy, and safety.

Literature review

Literature review

1. Galangal (*Alpinia galanga*)

Alpinia galanga (L.) Willd. commonly known as galangal (Khorjlan or Khulanjan) in different regions, is a rhizomatous plant from the Zingiberaceae family and the *Alpinia* genus (Fig. 1) (Zhang et al., 2021). It is widely used as a culinary spice and in traditional medicine (Verma et al., 2011). It is cultivated in regions with abundant sunlight and a humid climate. Indonesia is the largest producer and international supplier of, where it is widely cultivated (Trimanto et al., 2021d). In addition to Indonesia, *A. galanga* is also grown in several Asian countries, including India, China, Malaysia, Egypt, Sri Lanka, and Thailand (Gupta et al., 2014b).



Figure 1. *Alpinia galanga* wild rhizome (Hebbbar, 2023).

The rhizome of *A. galanga* is used for various medicinal purposes, it helps treat conditions like heart disease, diabetes, rheumatic pains, and chest pain. Additionally, it improves appetite, acts as an aphrodisiac, expectorant, and disinfectant (Verma & Sharma, 2022). The rhizomes of *A. galanga* are naturally packed with beneficial compounds, including phenolic substances such as phenylpropanoids, lignans, flavonoids, and essential oils. These elements contribute to its strong therapeutic potential and traditional medicinal use (Ramanunny et al., 2022).

It contains a variety of bioactive compounds in all parts of the plant with antimicrobial, antioxidant, anti-ulcer, antibacterial, antitumor, anti-HIV, antifungal, anti-inflammatory, anti-allergic, and antidiabetic properties (Trimanto et al., 2021).

2. Ethnopharmacology and drug discovery

According to the WHO, many rural communities in developing countries, around 80%, still rely on traditional medicine, passed down through generations. Today, there's a growing effort to bring these practices together with modern healthcare, aiming for a more inclusive and holistic approach to medicine (Coumaré 2021).

Ethnopharmacology is a multidisciplinary scientific field that explores how different cultures use natural substances, whether from plants, animals, or minerals, along with traditional knowledge and practices, to treat, prevent, or diagnose health conditions (Chaachouay et al., 2020).

Besides, medicinal herbs continue to be used extensively in medicine, owing to their efficacy, affordability, availability, and tolerance by the patient, despite the discovery of modern synthetic drugs (Sohani 2019). An estimated 25% of synthetic drugs are plant-derived either directly or indirectly (Jamshidi-Kia et al., 2018). Therefore, ethnopharmacological and ethnomedicinal research play a vital role in uncovering precious local knowledge and natural remedies, helping to preserve the rich traditions of medicinal culture for future generations (Taïbi et al., 2020).

Ethnopharmacology plays a crucial role in modern drug discovery by providing a foundation for identifying bioactive compounds from traditional medicinal practices. Indigenous knowledge systems offer valuable insights into the therapeutic uses of plants, animals, and minerals, which can guide researchers in selecting potential candidates for pharmacological investigation (Heinrich & Jäger, 2015). For instance, many widely used drugs, such as aspirin (derived from willow bark), morphine (from opium poppy), and quinine (from cinchona bark), have their origins in traditional medicine (Fabricant & Farnsworth, 2001).

Moreover, ethnopharmacology facilitates the discovery of novel chemical structures with unique mechanisms of action. Traditional healers often use complex mixtures of natural substances, which may exhibit synergistic effects that enhance therapeutic outcomes (Wagner & Ulrich-Merzenich, 2009). Modern pharmacological studies can isolate and validate these active compounds, leading to the development of new drugs for conditions such as cancer, malaria, and chronic inflammatory diseases (Cragg & Newman, 2013). For example, the antimalarial drug artemisinin was discovered through the study of traditional Chinese medicine, specifically the use of *Artemisia annua* (Tu, 2011). This highlights how ethnopharmacology bridges traditional knowledge and contemporary medicine, fostering innovation in pharmaceutical research.

3. Bioactive compounds of aromatic and medicinal plants

Medicinal plants are used for their therapeutic properties, due to their various compounds offering different health benefits. According to Gurib-Fakim (2006) and Singh (2015), people use herbal remedies made from all sorts of plant parts, whether it's leaves, stems, roots, seeds, fruits, bark, or even the entire plant. These parts contain natural compounds that can help heal in different ways, either directly or indirectly.

Indeed, plants can synthesize a large class of valuable organic compounds necessary for their growth and upkeep. Such necessary metabolites are nucleic acids, carbohydrates, lipids, and proteins, which serve central roles in sexual reproduction and in cellular processes. Secondary metabolites in plants come from primary metabolic products but are found in fewer species. While some clearly serve important roles like defense, coloring, or attracting pollinator. However, for many others, we still don't fully understand what they do or how they benefit the plant (Russell and Duthie, 2011). These compounds are usually sorted into three main groups: terpenoids, phenolic compounds, and alkaloids (Kabera et al., 2014).

3.1. Terpenoids

Terpenoids, with over 40,000 identified structures, comprise one of the broadest categories of natural products. They are particularly prevalent in plants, where they serve vital functions in growth and development regulation, pathogen and pest defense, and environmental communication (Schrader & Bohlmann, 2015). Terpenoid biosynthesis primarily involves the condensation of five-carbon isopentenyl pyrophosphate molecules. They are recognized for their many medicinal applications, which include antihypertensive, antimicrobial, and insecticidal activities (Kabera et al., 2014). Because of these properties, terpenoids play a significant role in the human diet and are commonly used as medicines or dietary supplements to help prevent or protect against various diseases, including cancer (Jamwal et al., 2018).

3.2. Phenolic compounds

Phenolic compounds, mostly found in plants, come in a wide variety of structures. They can be attached to other molecules or exist freely, either as simple units (monomers) or linked together in more complex chains (polymers) (Alara et al., 2021). These molecules offer several health benefits, including antimutagenic, antioxidant, anticancer, and anti-inflammatory effects (Ganaie, 2021). The most significant among these beneficial compounds are phenolic acids, flavonoids, and tannins (Vuolo et al., 2019).

3.2.1. Flavonoids

Flavonoids make up a large and diverse group of natural compounds, with over 10,000 different types identified to date (Fig.2). They, especially quercetin, are powerful natural compounds with antioxidant, anti-inflammatory, anti-allergic, antibacterial, antiviral, antiplatelet, and antineoplastic effects (Teoh, 2016).

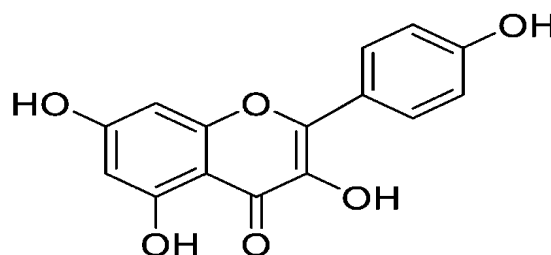


Figure 2. Structure of flavonoids (Ullah et al., 2020).

3.2.2. Phenolic acids

Phenolic acids are widespread plant secondary metabolites that contribute to the flavor, taste, and health benefits of fruits and vegetables (Fig. 3). They play a vital role in plant growth, reproduction, and defense against environmental stressors such as light, cold, and pollution (Ghasemzadeh, 2011). These compounds are produced via the phenylpropanoid pathway and exhibit various biological activities, including antidiabetic, antioxidant, anticancer, antimicrobial, and neuroprotective effects (Kumar and Goel, 2019).

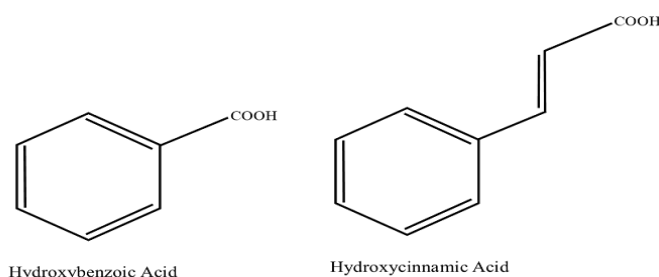


Figure 3. Structure of phenolic acids (Ali et al., 2020).

3.2.3. Tannins

According to Sieniawska (2015b), tannins are a type of phenolic compound with molecular weights typically ranging from 500 to 3000 Daltons (Da), making them relatively large and complex molecules. They are known for their powerful health benefits., including anti-inflammatory, antimicrobial, antioxidant, anticancer and anti-biofilm effects (Pizzi, 2021c).

These properties make them effective in fighting oral bacteria, reducing gum inflammation, and protecting tissues from oxidative stress (Kováč et al., 2022). There are two categories of tannins: (Fig. 4)

- Hydrolysable tannins: composed of polyhydric alcohols with hydroxyl groups esterified by gallic acid or similar compounds; they can be broken down by hydrolysis.
- Condensed tannins: formed by the condensation of phenolic compounds; they are non-hydrolysable and also known as proanthocyanidins (Ghosh 2015).

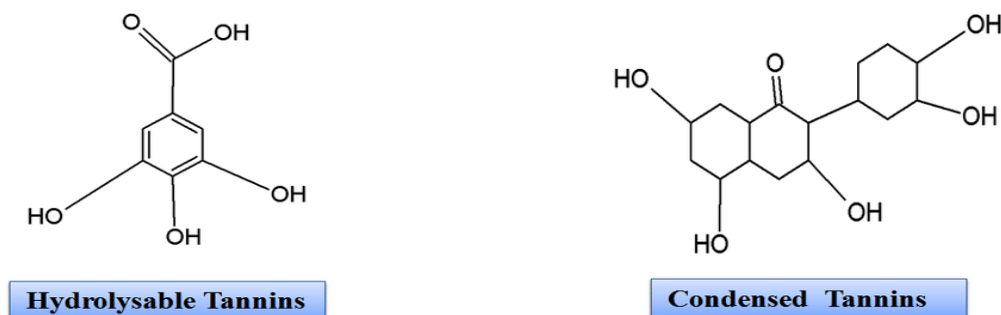


Figure 4. Structure of tannins (Falavinha et al., 2025).

3.3. Alkaloids

Alkaloids are among the largest groups of specialized metabolites found in both animals and plants, playing a key role in the development of pharmaceuticals derived from natural products (Zhang et al., 2022). They are diverse natural compounds valued for their therapeutic effects, such as anti-inflammatory, antitumor, and antimicrobial activities (Rosales et al., 2020). Thus, they have been used to treat various health issues, offering effects such as cough relief, calming (sedative) actions, and purgative (cleansing) properties (Yang and Stöckigt, 2010). It is mainly biosynthesized from amino acids like tyrosine, lysine, tryptophan, and aspartic acid, as it serves a protective role against herbivores and pathogen attacks, their structure and function vary depending on the plant species that produce them (Ballout et al., 2019). Alkaloids fall into three main categories based on how they're made and where their nitrogen atoms come from (Kukula-Koch & Widelski, 2016):

- True alkaloids: are directly made from amino acids and include a nitrogen atom in a ring-like structure. Well-known examples are “atropine” and “nicotine” (Zandavar & Babazad, 2023).
- Proto-alkaloids: also come from amino acids, but their nitrogen atom isn't part of a ring. Some familiar ones include “taxol” and “ephedrine” (Zandavar & Babazad, 2023).
- Pseudo-alkaloids: on the other hand, don't come from amino acids at all. Instead, they're made in other ways and include popular substances like “caffeine” and “theobromine” (Zandavar & Babazad, 2023).

4. Toxicity of aromatic and medicinal plants

Certain medicinal plants may be harmful since their side effects can interfere with the body's normal functions and negatively impact health (Oyedare et al., 2009). The World Health Organization has documented close to 5,000 negative reactions caused by plants, and the US Food and Drug Administration has reported over 2,600 side effects from dietary supplements—tragically including 101 deaths (Subramanian et al., 2018).

4.1. Cell cycle and cell fate

The cell cycle is the series of stages through which a cell replicates its DNA and divides into two identical daughter cells (Fig. 5) (Yang, 2018):

1. Interphase: During interphase, chromosomes are mostly in a decondensed state, yet chromatin remains organized in a non-random manner within the nucleus. Each chromosome resides in a distinct and specific nuclear region called a chromosome territory (Tiang et al., 2011).
2. Prophase: During prophase, the nuclear envelope disintegrates and organelles disperse, while the nucleolus disappears. Centrosomes move to opposite poles and form the mitotic spindle, as sister chromatids condense and become visible under the microscope (Bartee, 2019).
3. Metaphase: During metaphase, all chromosomes align along the metaphase plate, positioned midway between the cell's two poles. At this stage, the sister chromatids remain firmly connected by cohesin proteins, and the chromosomes reach their highest level of condensation (Bartee, 2019).
4. Anaphase: During anaphase, sister chromatids separate and move toward opposite poles, while the cell elongates due to the sliding of polar microtubules (Bartee, 2019).
5. Telophase: During telophase, chromosomes arrive at opposite poles and begin to decondense into chromatin. The mitotic spindle disassembles, and new nuclear envelopes form around each set of chromosomes, with nucleoli reappearing (Bartee, 2019).

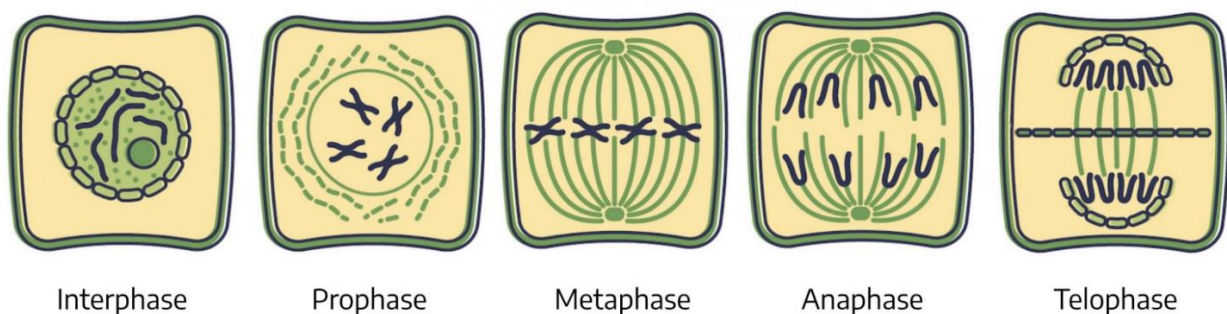


Figure 5. Cell cycle (Michaels et al., 2022).

Cell death is a biological process that results in the cessation of cell function and, eventually, cell death. Apoptosis, necrosis, and autophagy are three distinct forms of cell death, each with unique mechanisms and biological roles (Fig. 6) (Park et al., 2023).

Apoptosis is the body's natural way of removing cells that are damaged or no longer needed, through a carefully controlled process. During apoptosis, cells shrink, their DNA breaks down, and they split into small parts that the immune system cleans up quietly without causing harm or inflammation (Park et al., 2023).

Necrosis is an uncontrolled form of cell death triggered by severe damage, such as lack of oxygen to the brain. It leads to cell swelling, organelle damage, membrane rupture, and the release of cell contents, often causing inflammation in surrounding tissues (Hu & Liu, 2017).

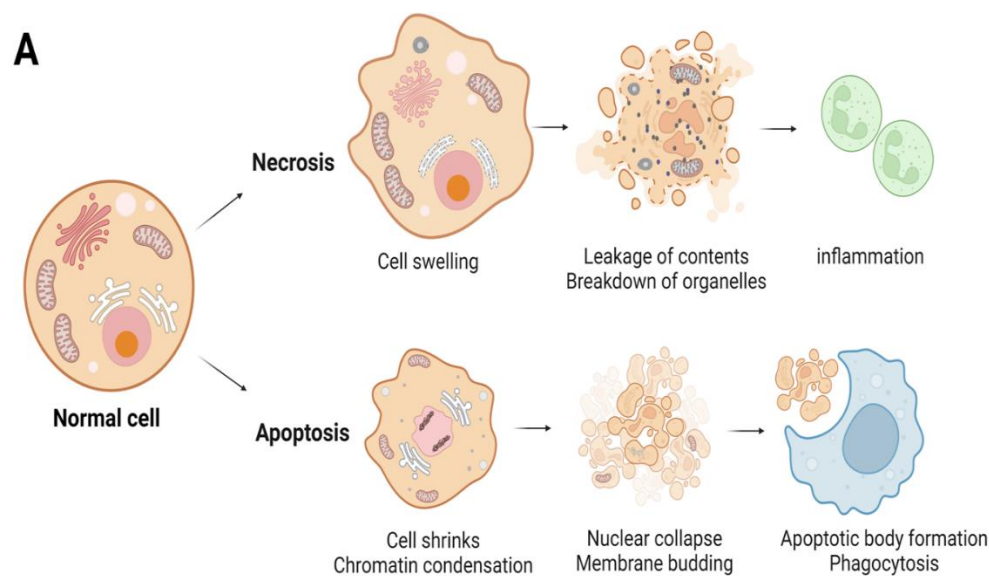


Figure 6. Necrosis and apoptosis: morphological features (Park et al., 2023)

Autophagy is like the cell's natural cleanup crew, helping to clear out damaged or worn-out parts so the cell can stay healthy and function properly (Fig. 7). It becomes especially important when the cell is under stress. This process supports everything from development to immune defense and aging. When autophagy doesn't work well, it can contribute to diseases like cancer or brain disorders (Istifli et al., 2019).

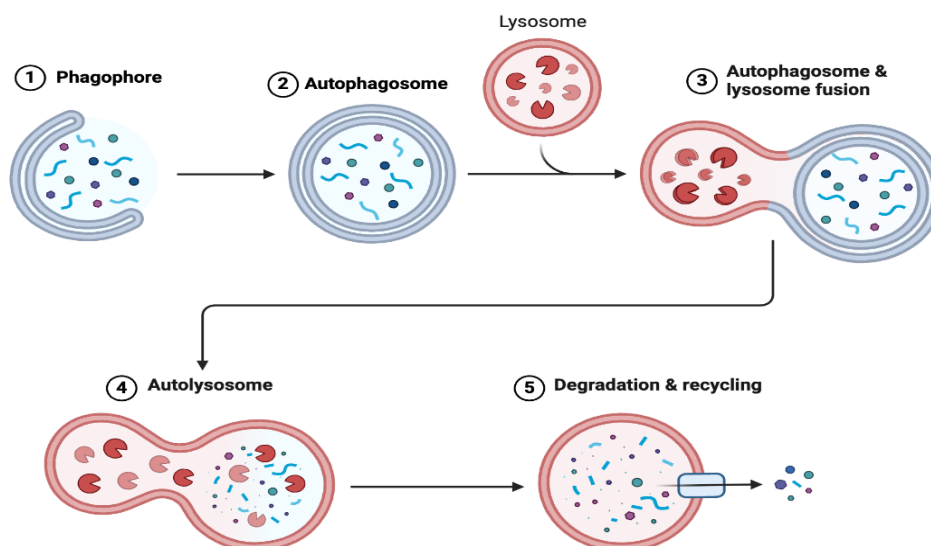


Figure 7. Autophagy (Autophagy Process | BioRender Science Templates, s. d.)

4.2. Cytotoxicity

Cytotoxicity describes the extent to which a substance can damage or kill living cells. It helps scientists understand how harmful a substance might be to the body at the cellular level, making it crucial for assessing the safety of medications and other products (Lim et al., 2021). It can be triggered by various agents such as chemicals, plant extracts, or pharmaceuticals (Zhang, 2018). Chemical substances are generally known as cytostatic agents because they slow down or stop cell division. Physical factors like elevated temperatures, ultrasonic waves, and radiation can also harm cells and disrupt their function (Tülay Aşkin, 2018). On the biological side, harmful agents may come from bacteria, viruses, animals, or plants—such as antibiotics or the toxins bacteria release, including both endotoxins and exotoxins (Herdiana et al., 2021).

Cytotoxicity assays are essential for quickly evaluating whether plant-derived substances may be harmful to cells (Çelik 2018). The most commonly used test is the MTT or XTT assay, which evaluates how alive and active cells are by measuring their metabolic activity. This method gives insight into how a substance might affect cell health and survival (Istifli et al., 2019). The Neutral Red cytotoxicity (NR) assay is a method designed to evaluate cell function by assessing the integrity of lysosomal membranes (Liu et al., 2018). *Allium cepa* test serves to detect mitotic cycle disturbances and inhibit root growth, providing insights into cytotoxic and genotoxic effects (Cabuga et al 2017).

4.2.1. Evaluation of cytotoxicity using MTT assay

The MTT assay (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) (and to a lesser extent MTS, XTT, or WST assays) is a commonly used method for evaluating cell viability

and assessing the cytotoxic effects of drugs (Stepanenko and Dmitrenko, 2015). The MTT assay protocol begins by seeding 500 to 10,000 cells per well in a standard 96-well plate. After allowing the cells to adhere and grow, the MTT reagent is added. Living cells convert the yellow MTT dye into insoluble purple formazan crystals through mitochondrial enzyme activity. These crystals are then solubilized, and the resulting solution's absorbance is measured at 570 nm using a spectrophotometer. This absorbance correlates with cell viability. The assay is suitable for both adherent and suspension cells and can be scaled to other plate formats if needed (Kumar et al., 2018).

3.2.2. Evaluation of cytotoxicity using NR assay

Cells are incubated with the Neutral Red dye, which is taken up by the lysosomes of viable cells. After an incubation period (usually around 2 hours), the dye solution is removed, and the cells are washed. The accumulated dye is then extracted using a desorption solution. The absorbance of this extracted dye is measured spectrophotometrically to assess cell viability. This assay is simple, sensitive, and widely used to evaluate the toxicity of substances on cultured cells (Zhang et al., 1990).

4.3. Genotoxicity

Genetic information, chemically encoded in DNA, is maintained, replicated, and transmitted to successive generations with high fidelity. DNA damage can occur through normal biological processes or as a result of interactions—either direct or indirect—with chemical, physical, or biological agents (Young, 2002). These agents can affect cells either directly by interacting with DNA in chromatin or chromosomes, or indirectly by targeting proteins involved in DNA replication or maintaining chromosome stability (Fig. 8) (Radhika et Jyothi 2019).

Genotoxicity tests help detect whether a substance can damage genetic material, even in small amounts. If such substances interact with DNA, they can cause mutations or chromosomal changes that may alter how the cell functions, sometimes leading to lasting and harmful effects (Sponchiado et al., 2015b).

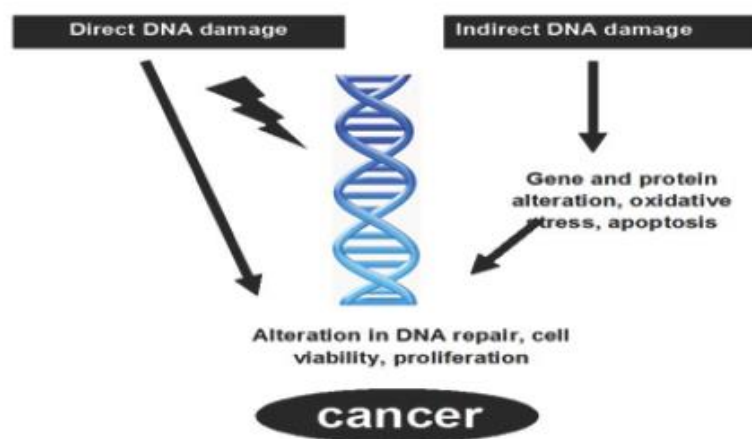


Figure 1. genotoxicity damages (Sv, 2017).

4.3.1. Evaluation of genotoxicity using Ames test

The bacterial reverse mutation test, also known as the Ames test, is the most widely used method for detecting genotoxicity. It serves as a key part of chemical safety evaluations required by regulatory bodies across the globe (Madia et al., 2020). This test uses mutated strains of *Salmonella* and *E. coli* that need histidine to grow. When exposed to mutagens, some bacteria revert to grow without histidine, forming colonies. An increase in these revertant colonies indicates DNA damage caused by the mutagen (Zeiger, 2019).

4.3.2. Evaluation of genotoxicity using Comet test

The comet assay is a flexible technique used to detect nuclear DNA damage in individual eukaryotic cells, ranging from yeast to humans (Collins et al., 2023). According to Salimraj et al. (2025) The test involves first lysing the cell membranes, then denaturing the DNA in a strongly alkaline environment. Next, the DNA is subjected to a low-voltage, low-amperage electric field, allowing the damaged, shorter DNA fragments to migrate out of a compact sphere formed by intact DNA. This migration creates a stretched "halo" of DNA toward the anode, revealing the presence of DNA damage.

4.3.3. Evaluation of genotoxicity using *Allium cepa* test

Allium cepa (common onion) is widely considered a reliable genetic model for identifying potential genotoxic substances present in the environment (Firbas and Amon 2014) due to their rapid growth, easy manipulation, sensitivity to toxic substances, and large, clearly visible chromosomes with a reduced number ($2n = 16$), making them ideal for observing genetic damage

(Rosculete et al. 2019). The results of the *Allium cepa* test are consistent and comparable to those from tests conducted on animal cell lines (Herrero et al., 2012) (Fig. 9).

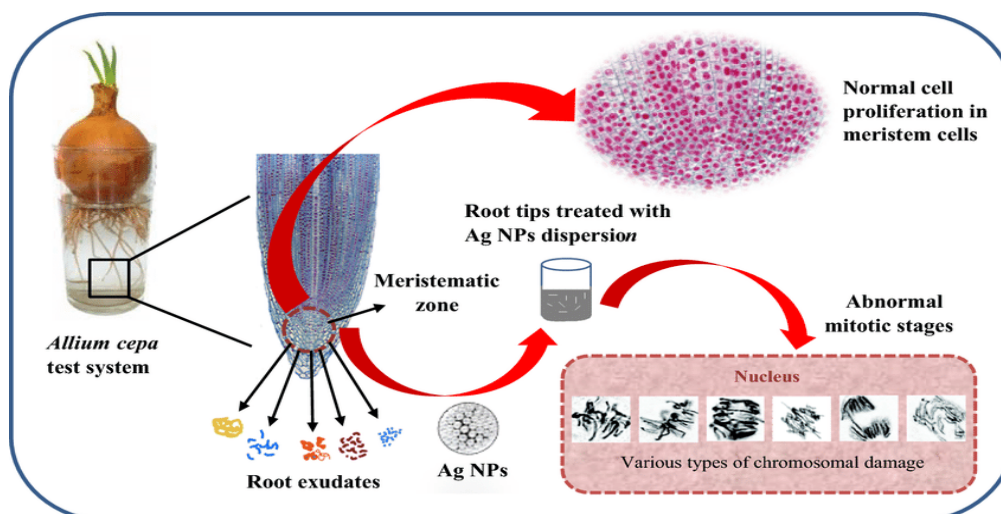


Figure 9. *Allium cepa* test (Vinay et al., 2019).

Methodology

Methodology

1. Ethnopharmacological study

1.1. Region of study

This ethnopharmacological study was conducted in various regions of Algeria, specifically, Tiaret, Ourgla, Ghardaia, Mila, Bordj bouaareridj, Blida, Alger, Tissemsilt, Ain defla, Biskra, Guelma, Batna, Bechar, Setif, Msila, Djelfa to explore and document the various traditional uses of *Alpinia galanga* in their daily life.

1.2. Conduct of the study

The present ethnopharmacological study took place during the period from February to June 2025 on a total of 200 participants living in rural or urban areas and occupying different functions i.e., local traditional healers, herbalists, elderly individuals known for their expertise in medicinal plant use among others. Each participant was interviewed individually.

As this study aims to identify and collect the different uses of *A. galanga* in traditional medicine in Algeria, all the information has been documented while indicating the parts used, the method of preparation, the routes of administration of the recipes and their dosage. Participants were also asked for the plant toxicity.

1.3. Data collection

Data collection was carried out using a pre-established semi-structured questionnaire comprising two main parts; the first part concerns the participants (sex, age, function, school level, place and living environment). However, the second part concerns information relating to the traditional uses of *A. galanga*. This information makes it possible to identify the part used and to assess the duration of its use, the reasons for its use, its toxicity and the recommended method of preparation, among other details. The participants were questioned using the local dialect, searching after the survey for the origin of this knowledge and the description of the cited product in order to facilitate its identification later.

1.4. Data processing and analysis

The information gathered was categorized in Excel file to compute the frequency of use or citation of the listed natural products.

2. Phytochemical study

2.1. Plant collection

The rhizomes of galangal were purchased from the local market and 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.

2.2. Preparation of plant extracts

In a glass flask, 50 g of plant powder and 500 mL of distilled water were macerated to prepare the aqueous extract. For a whole day, the mixture was left in the dark at room temperature while being stirred with a magnetic stirrer. After the extract was filtered through Whatman paper (Fig. 10), the filtrate was dried in an incubator set at 37 °C to produce a dry residue, which was thereafter placed in a sterile box for storage.



Figure 10. Procedures of Preparation of plant extracts

33.5 g of powder was added to 335 mL of 70% ethanol in a glass flask to get the hydroethanolic extract. The mixture was macerated for twenty-four hours. Whatman paper was used to filter the resultant extract, and the filtrate was then dried in an incubator set at 37 °C to produce a dry residue that was stored away in a sterile box.

Additionally, a simple infusion of *A. galanga* was prepared to simulate traditional usage practices. A quantity of plant powder was added to boiling distilled water (According to the recommended concentration by local population). The mixture was left to steep for about 15 to 20 minutes, then allowed to cool at room temperature.

2.3. Evaluation of phytochemical compounds

2.3.1. Polyphenols

To evaluate the total phenolic content, 200 μL of each extract (both aqueous and hydroethanolic), at concentrations of 0.25, 0.5, 1, 1.5 and 2 mg/mL, and infusion at concentrations of 2.5, 5, 10 and 20 mg/mL; was carefully pipetted into Eppendorf tubes. We then added 1 mL of Folin–Ciocalteu reagent, previously diluted tenfold, to each sample. The tubes were kept in the dark at room temperature for 5 minutes to prevent light interference. After that, 800 μL of a 7.5% sodium carbonate solution was added, and the tubes were gently shaken to ensure proper mixing.

They were then left at room temperature for 1 hour to allow the reaction to take place. Absorbance was measured at 765 nm using a spectrophotometer. The total phenolic content was calculated based on a gallic acid standard curve and expressed as milligrams of gallic acid equivalent (GAE) per milliliter of extract. To ensure the reliability of the results, each test was performed at least three times.

2.3.2. Flavonoids

To measure total flavonoid content, 1 mL of each plant extract was mixed with 1 mL of a 2% aluminum chloride (AlCl_3) solution in methanol, in an Eppendorf tube. The mixture was then shaken thoroughly with a vortex and left to incubate in the dark for 15 minutes. After that, the absorbance was measured right away at 430 nm, using distilled water as a blank. The flavonoid levels were calculated based on a standard calibration curve and expressed as milligrams of quercetin equivalent (QE) per milliliter of extract. Each test was carried out at least three times to ensure reliability.

2.3.3. Tannins

A volume of 50 μL from each extract was added to 1,500 μL of 49% vanillin in methanol and mixed vigorously. Subsequently, 750 μL of concentrated hydrochloric acid (HCl) was added. The mixture was left to react at room temperature for 20 minutes. Absorbance was then recorded at 550 nm using a blank for reference. Total tannin content was evaluated using a standard calibration curve and reported as milligrams of tannic acid equivalent (TAE) per mL of extract. All tests were carried out in triplicate to ensure reliability.

3. Cyto-genotoxicity study

3.1. *Allium cepa* bioassay

Allium cepa ($2n = 16$), the onion plant, a member of the Alliaceae family. Based on their size, onion bulbs were bought from a Tiaret, Algeria, local market. After the roots and first peel were removed, the bulbs were cleaned in distilled water. Before starting the *Allium* anaphase-telophase test, the dry bottom plate and bulb outer scales were taken off without damaging the root primordia. over this investigation, bulbs with roots that ranged in length from 1 to 2 cm were exposed to distilled water over three days in the dark at 21 ± 4 °C. This includes placing each bulb that has germinated on a test tube filled with distilled water, so that only the roots are immersed.



Figure 11. Procedures of *A. cepa* assay.

The mitotic index (MI) (%) = Number of dividing cells (P+M+A+T) / Number of cells counted x 100.

The Phase Index (PI) (%) = Number of cells in specific phase/ Number of dividing cells x 100.

The Chromosomal Aberrations Index (CA) (%) = Number of aberrant cells/ Number of cells counted x 100.

Results

Results

The results of this study are presented in three main parts: the ethnopharmacological investigation, the phytochemical analysis, and the evaluation of cytotoxic and genotoxic effects of *A. galanga*. Each section highlights the key findings obtained through interviews, laboratory analyses, and biological assays. The data provide a comprehensive understanding of the traditional uses, chemical composition, and biological safety of the plant, supporting its potential therapeutic value while identifying possible toxicological concerns.

1. Ethnopharmacological study

1.1. Characterization of the participants

The age distribution of the participants indicates that individuals between 51 and 55 years old were the most represented group, with 40 occurrences. This was closely followed by those aged 46–50 and 56–60, each with 35 participants. Other age groups such as 41–45 and 61–65 were also notably represented with 30 individuals each. Participation gradually decreased among younger and older age groups, with 25 people in both the 36–40 and 66–70 brackets, and lower counts among the youngest (under 25: 10) and oldest (76 and over: 10). Overall, the data show that middle-aged adults formed the majority of the respondents (Fig. 12).

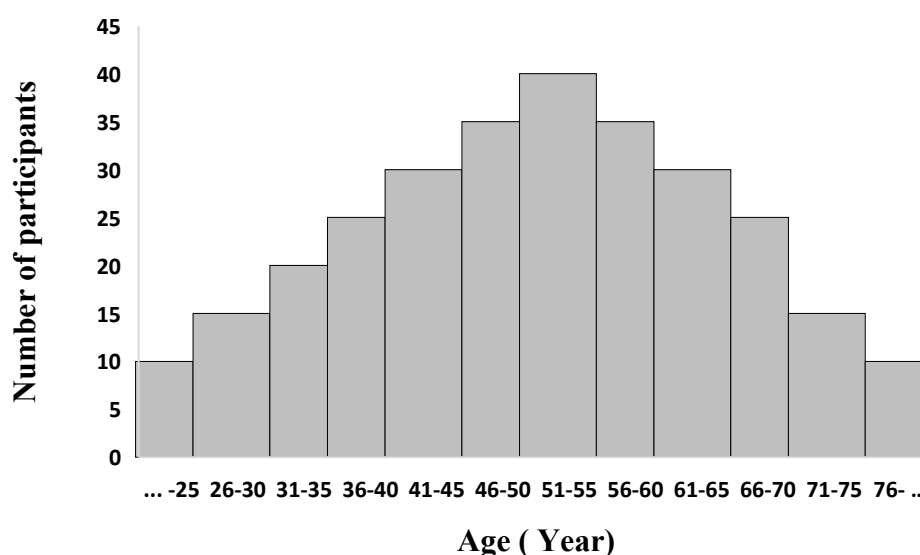


Figure 12. Distribution of the age groups of the participants.

The number of informants is almost evenly distributed between male and female, with 100 men (50 %) and 100 women (50 %) (Fig. 13).

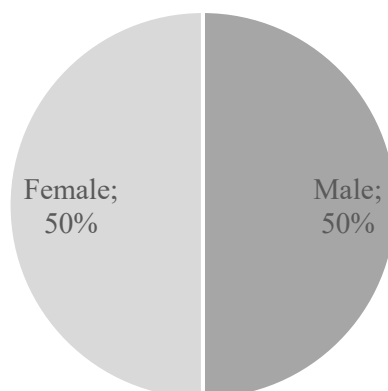


Figure 13. Distribution of participants by sex.

The majority of informants live in urban areas, with 160 people (80 %) compared to 40 people (20 %) living in rural areas. This indicates that information about *A. galanga* mainly comes from an urban population, which may reflect greater interest or access to the plant in urban settings compared to rural ones (Fig. 14).

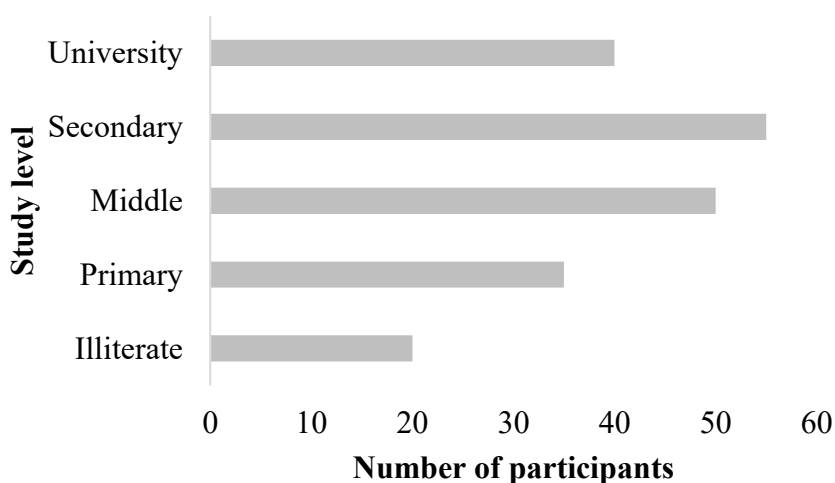


Figure 14. Level of study of participants.

Based on the provided data, the majority of participants had completed secondary education (55 individuals), followed by those with a middle school level (50) and university degree holders (40). Participants with only a primary education totaled 35, while the smallest group consisted of illiterate individuals (20). This distribution highlights that most respondents had at least a middle school education, with a notable representation from higher education levels as well (Fig. 15).

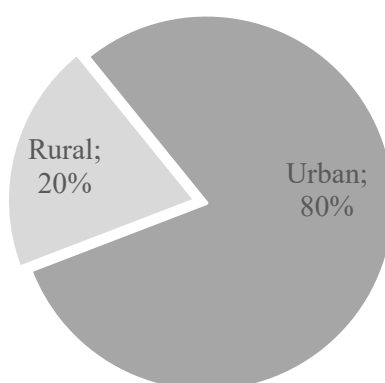


Figure 15. Distribution of participants according to their living environment.

The analysis of professional distribution of the participants reveals that the majority of respondents were herbalists (98 individuals), as they are considered to possess extensive traditional knowledge and maintain direct contact with patients. Other categories were also represented, including individuals working in the medical sector (55) i.e., doctors, pharmacists, midwives, nurses. Retired persons (35), housewives (5), unemployed individuals (4), and students (3) (Fig. 16).

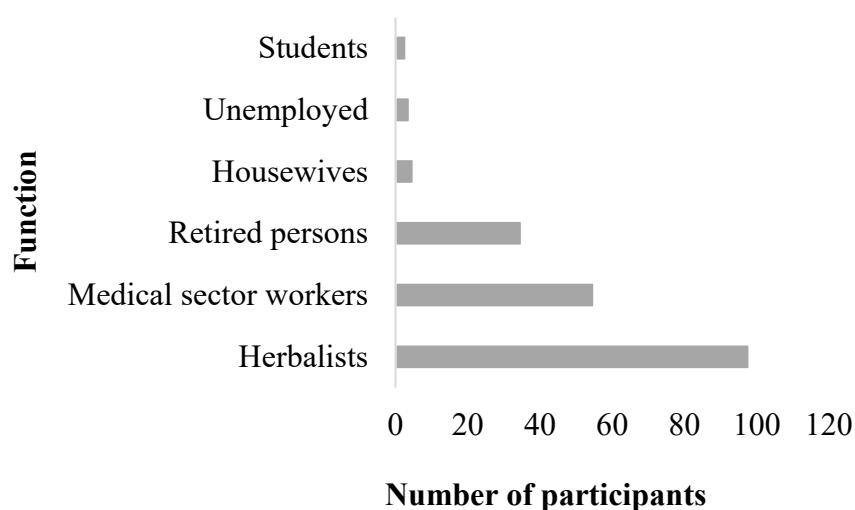


Figure 16. Nature of the function of the participants

The majority of informants consider that *A. galanga* is mainly considered unsafe for pregnant women (120 mentions). They do not recommend their uses also for breastfeeding women, cardiac, hypertensive, and diabetic patients. However, 45 informants do not report any restrictions (Fig. 19).

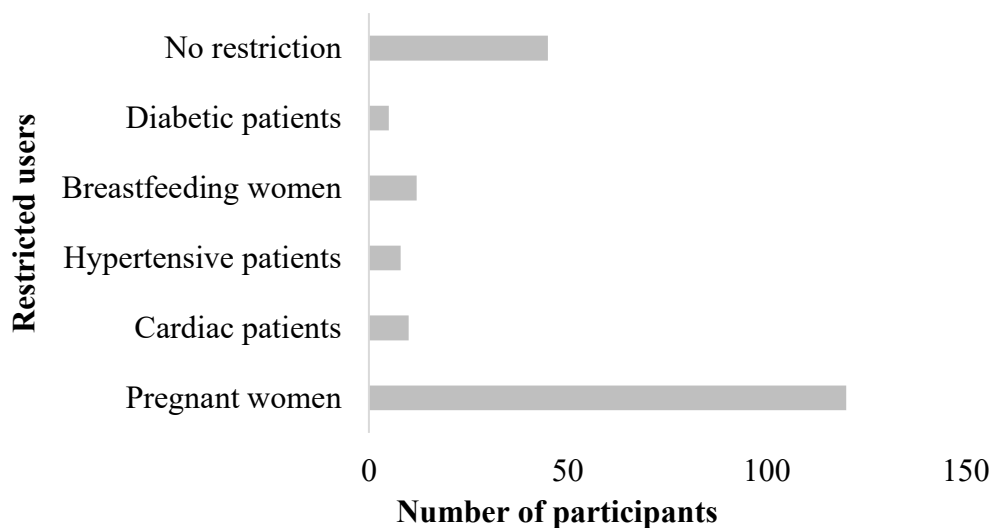


Figure 19. Restricted users of *A. galanga*.

2. Phytochemical study

2.1. Evaluation of the extraction yield

The extraction yield results indicate that the ethanolic extract of *A. galanga* provides a slightly higher yield (14.8 %) compared to the aqueous extract (13.8 %).

Table 1. Extraction yield of hydroethanolic and aqueous extracts.

Extract	Yield (%)
Aqueous extract	13,8
Ethanolic extract	14,8

2.2. Evaluation of the phytochemical compounds

2.2.1. Total phenolics content

The analysis of the data highlights a significant variation in total phenolic content between the aqueous and ethanolic extracts across the different concentrations tested (Fig. 20).

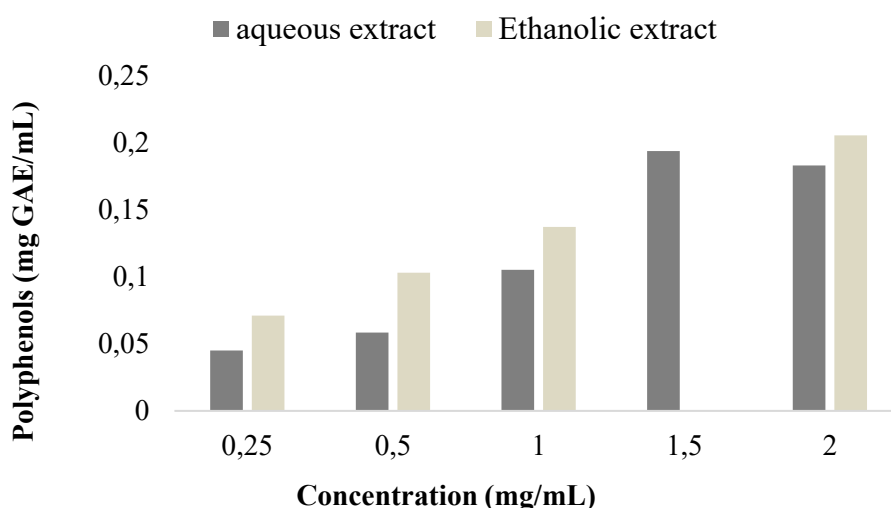


Figure 20. Variation of total phenolic contents in the aqueous and ethanolic extracts of *A. galanga*.

The ethanolic extract consistently exhibited a higher total phenolic content than the aqueous extract across most tested concentrations. The highest value was recorded at 2 mg/mL of ethanolic extract (0.205 mg GAE/mL), while the lowest was seen in the aqueous extract at 0.25 mg/mL (0.045 mg GAE/mL).

An exception occurred at 1.5 mg/mL, where only the aqueous extract was evaluated, showing a notably high content (0.193 mg GAE/mL), nearly comparable to that of the ethanolic extract at 2 mg/mL.

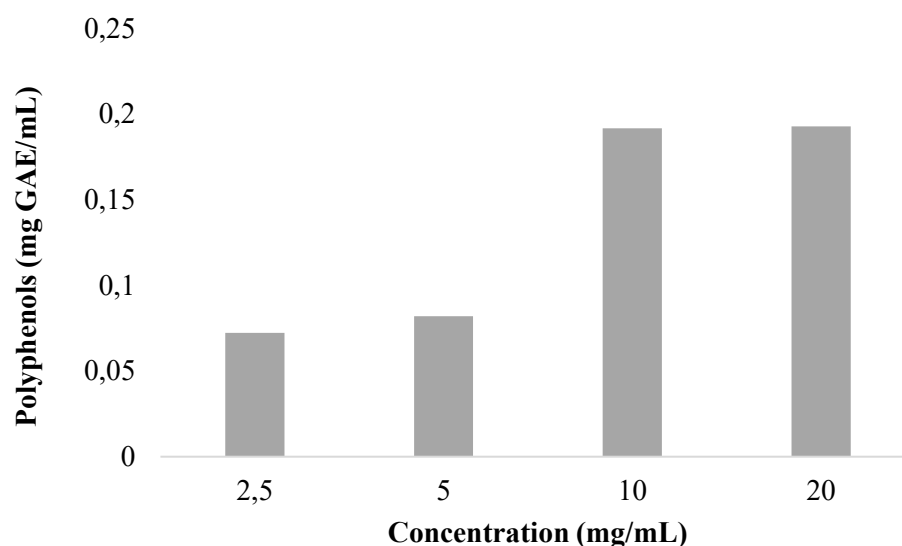


Figure 21. Variation of total phenolic contents in the infusion of *A. galanga*

Regarding the infusion, total phenolic content increased with concentration, peaking at 20 mg/mL (0.193 mg GAE/mL). These results suggest that ethanolic extraction is more efficient for phenolic compounds, though aqueous extracts and infusions also retain a significant amount, especially at higher concentrations (Fig. 21).

2.2.2. Total flavonoids content

By the same, the analysis of the data reveals a notable difference in the flavonoids content among aqueous and ethanolic plant extracts (Fig. 22).

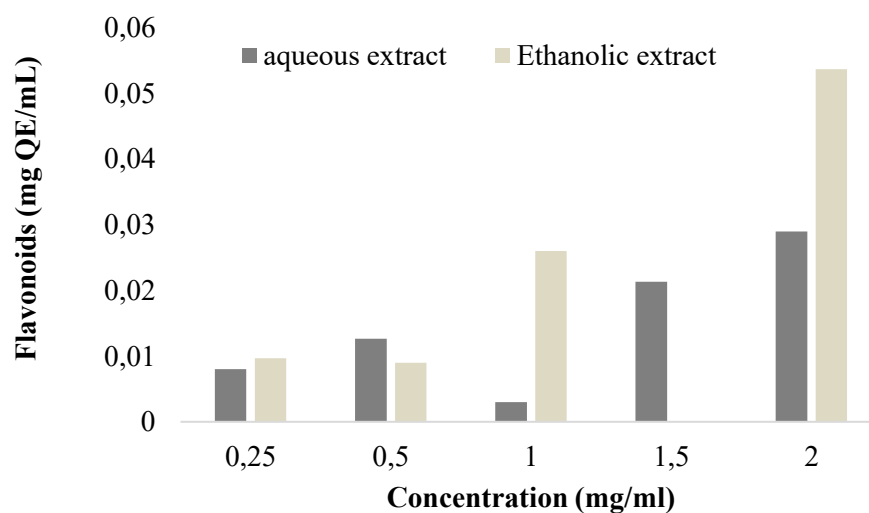


Figure 22. Variation of flavonoids contents in the aqueous and ethanolic extracts of *A. galanga*.

The data show that the ethanolic extract generally contained a higher level of total flavonoids compared to the aqueous extract across most concentrations. The highest flavonoid content in the ethanolic extract was recorded at 2 mg/mL (0.054 mg QE/mL), whereas the aqueous extract reached its peak at the same concentration with 0.029 mg QE/mL. Notably, at 1 mg/mL, the ethanolic extract showed 0.026 mg QE/mL, significantly higher than the aqueous extract at this concentration (0.003 mg QE/mL).

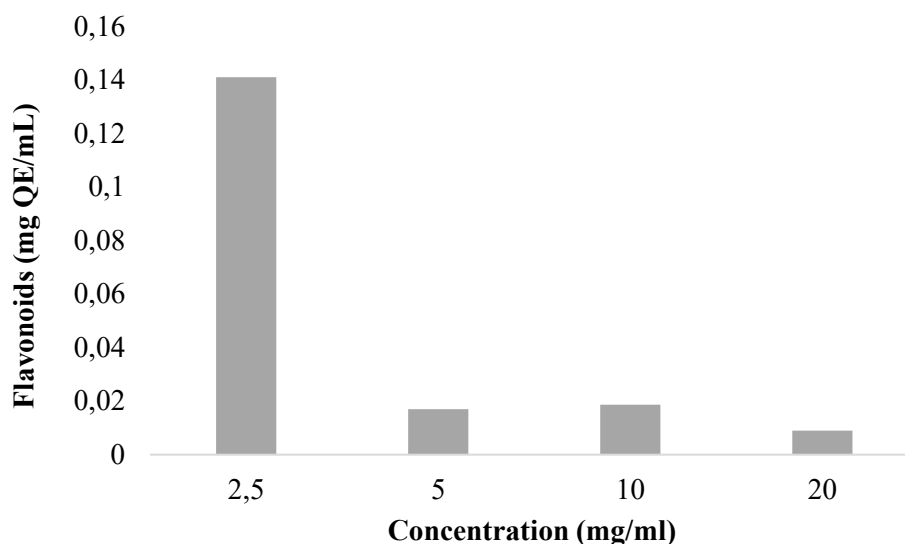


Figure 23. Variation of flavonoids contents in the infusion of *A. galanga*.

As for the infusion, the flavonoid content varied across concentrations, with a remarkably high value at 2.5 mg/mL (0.141 mg QE/mL), followed by a decrease at higher doses (Fig. 23).

2.2.3. Total tannins content

The content of tannins varied considerably between aqueous and ethanolic extracts of *A. galanga*, depending on the concentration (Fig. 24).

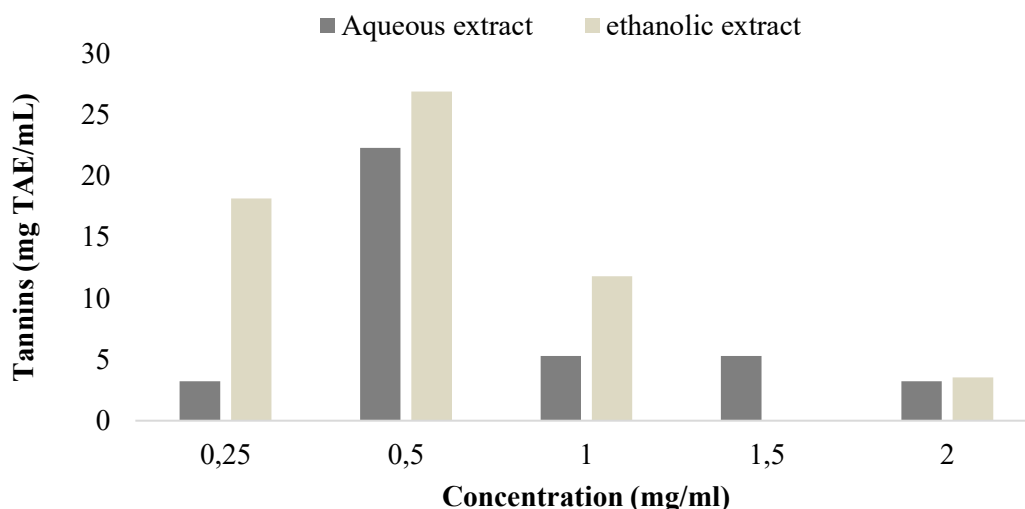


Figure 24. Variation of tannins contents in the aqueous and ethanolic extracts of *A. galanga*.

The aqueous extract showed its highest tannin content at 0.5 mg/mL (22.27 mg TAE/mL), with lower values at other concentrations, indicating optimal extraction at this level. In contrast, the ethanolic extract peaked at 0.5 mg/mL (26.87 mg TAE/mL) as well, but maintained relatively high tannin levels at 0.25 mg/mL (18.14 mg TAE/mL), then declined significantly at higher concentrations. Overall, both extracts were most effective at 0.5 mg/mL, but ethanolic extract demonstrated a slightly higher tannin yield at this concentration.

Infusion preparations demonstrated a progressive increase in tannin content with concentration (Fig. 25), ranging from 22.11 mg TAE/mL at 2.5 mg/mL to a maximum of 61.95 mg TAE/mL at 20 mg/mL, indicating a strong concentration-dependent extractive effect.

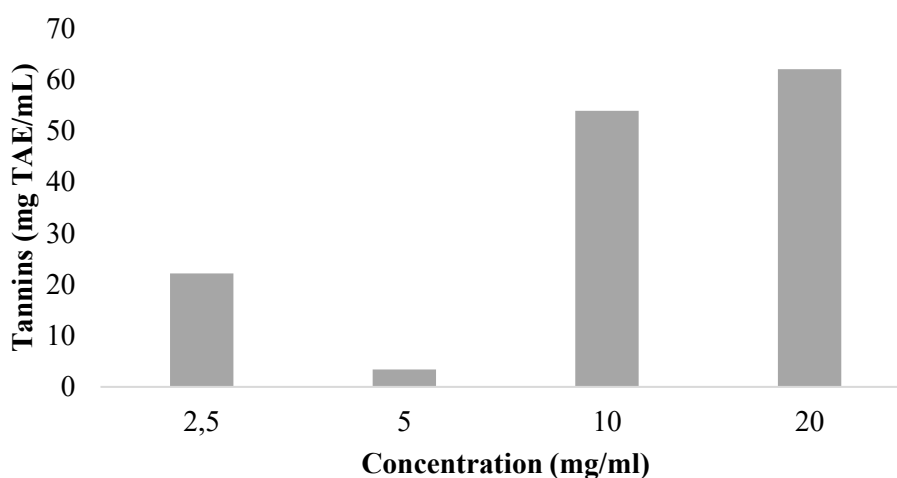


Figure 25. Variation of tannins contents in the infusion of *A. galanga*

3. Cyto-genotoxic study

3.1. Root morphometry

3.1.1. Roots number

The roots number of *Allium cepa* was recorded for each treatment prior to and following the incorporation of plant extracts and infusion into the growth medium. The positive control exhibited a rise from 21.25 to 26.5, and the negative control showed a more pronounced increase from 16.5 to 25.5, indicating that both controls supported root development.

The aqueous extract of *A. galanga* caused a slight increase in the number of *A. cepa* roots across all tested concentrations, with the most notable effect at 1 mg/mL (from 14.8 to 17). Other concentrations also showed minor increases, such as from 19.2 to 20.6 at 0.25 mg/mL and from 23.2 to 23.6 at 0.5 mg/mL (Fig. 26).

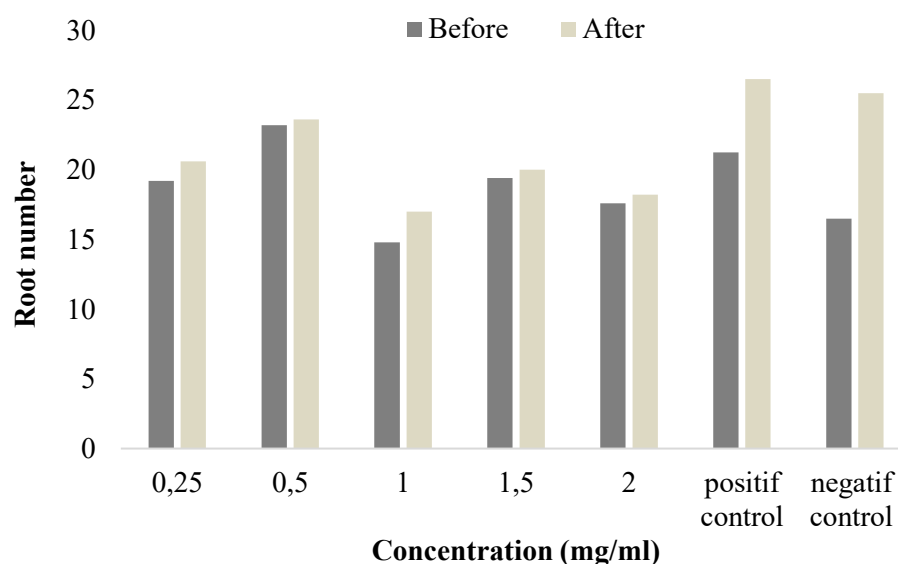


Figure 26. Roots number before and after treatment with aqueous extract.

The ethanolic extract of *A. galanga* showed a slight increase in the number of *A. cepa* roots at all concentrations except 0.5 mg/mL, where no change was observed. The most notable increase occurred at 0.25 mg/mL (from 12 to 14.4) (Fig. 27).

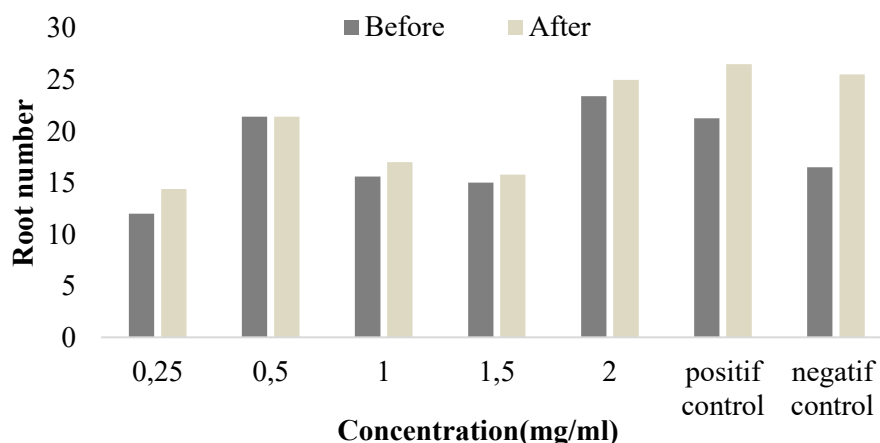


Figure 27. Roots number before and after treatment with ethanolic extract.

The infusion of *A. galanga* led to a moderate increase in the number of *A. cepa* roots across all tested concentrations. The most notable improvement was observed at 10 mg/mL, where root number increased from 10.25 to 16, and at 20 mg/mL, rising from 9.75 to 12.25. Lower concentrations such as 2.5 mg/mL and 5 mg/mL also showed slight increases (from 15.5 to 18.75 and from 19.25 to 20.5, respectively) (Fig. 28).

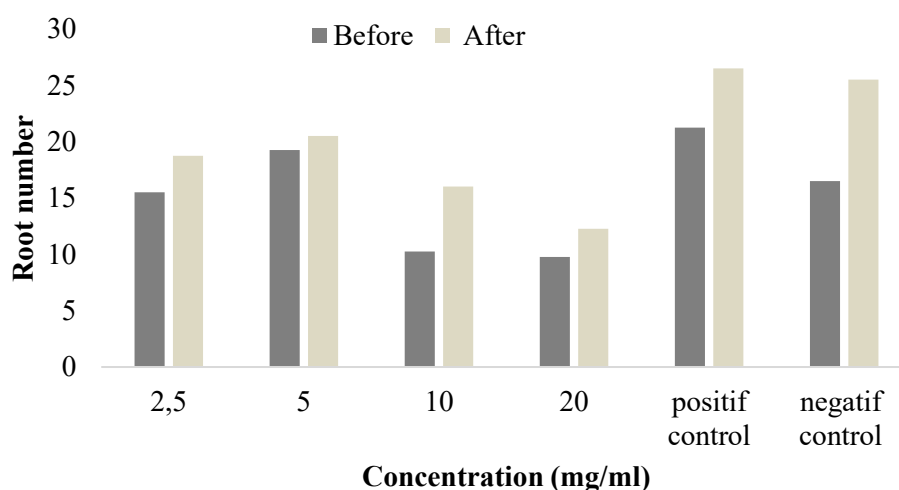


Figure 28. Roots number before and after treatment with infusion.

3.1.2. Roots length

The length of *A. cepa* roots was recorded also for each treatment prior to and following the incorporation of plant extracts and infusion. The aqueous extract of *A. galanga* stimulated the root growth of *A. cepa* in a dose-dependent manner, with the highest effect observed at 1 mg/mL (increasing from 27.12 mm to 33 mm), while a slight inhibition was noted at 2 mg/mL (decreasing from 29.85 mm to 28.25 mm). The positive control also showed a clear increase in root length (from 15.8 mm to 33.11 mm), and the negative control increased from 15.48 mm to 32.48 mm (Fig. 29).

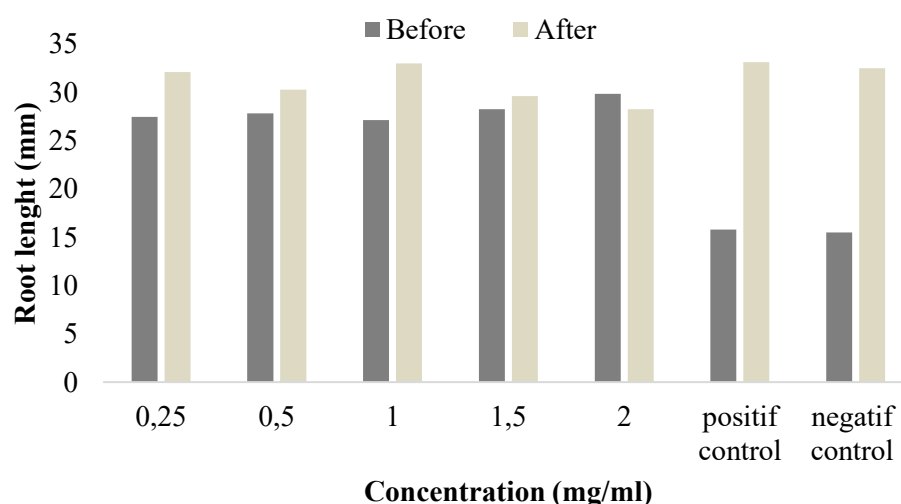


Figure 29. Roots length before and after treatment with aqueous extract.

The ethanolic extract of *A. galanga* produced variable effects on the root length of *A. cepa* (Fig. 30). A notable increase was observed at 0.5 mg/mL (from 28.71 to 33.4 mm) and 2 mg/mL (from 32.43 to 33.89 mm), indicating a stimulatory effect at these concentrations. In contrast, a decrease in root length was recorded at 1 mg/mL (from 32.43 to 24.86 mm) and 1.5 mg/mL (from 28.71 to 26.8 mm).

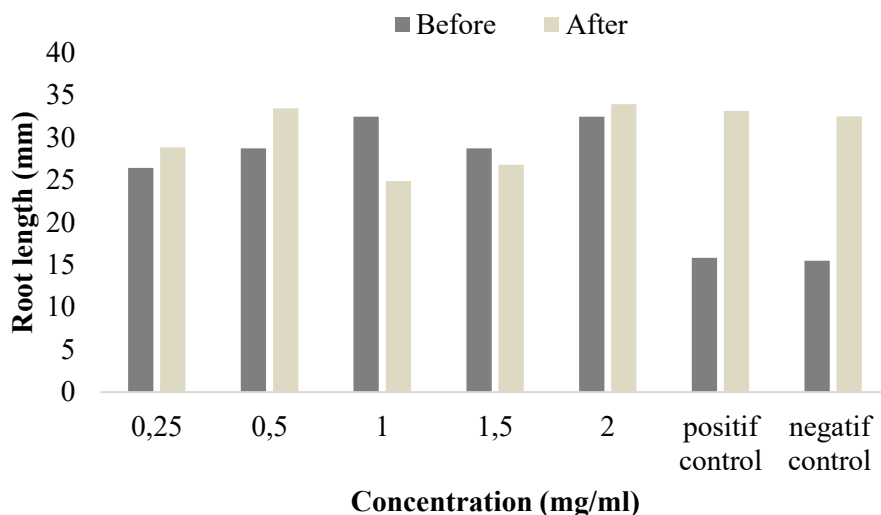


Figure 30. Roots length before and after treatment with ethanolic extract.

The infusion of *A. galanga* caused a noticeable increase in *A. cepa* root length across all tested concentrations (Fig. 31). The most pronounced effects were seen at 2.5 mg/mL (from 24.89 to 28.5 mm) and 10 mg/mL (from 19.56 to 23.39 mm), indicating a moderate stimulation of root growth. Even at the highest concentration (20 mg/mL), root length increased from 13.26 to 16.62 mm.

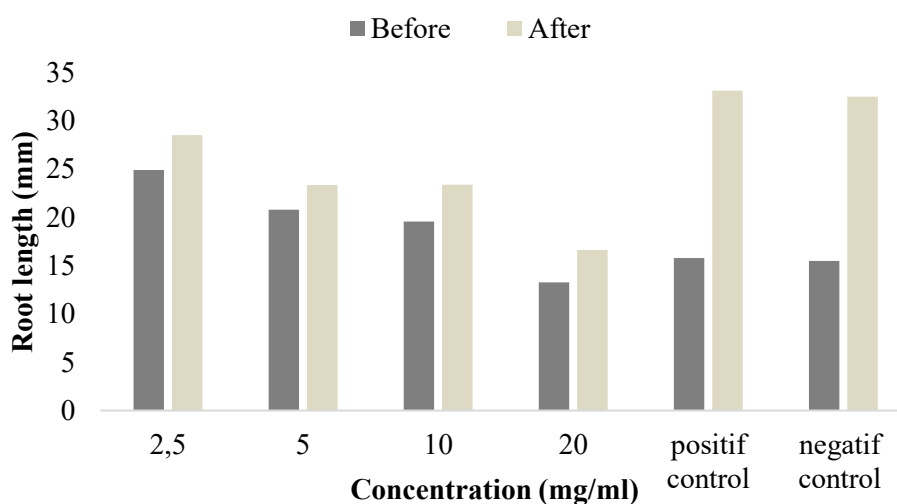


Figure 31. Roots length before and after treatment with infusion.

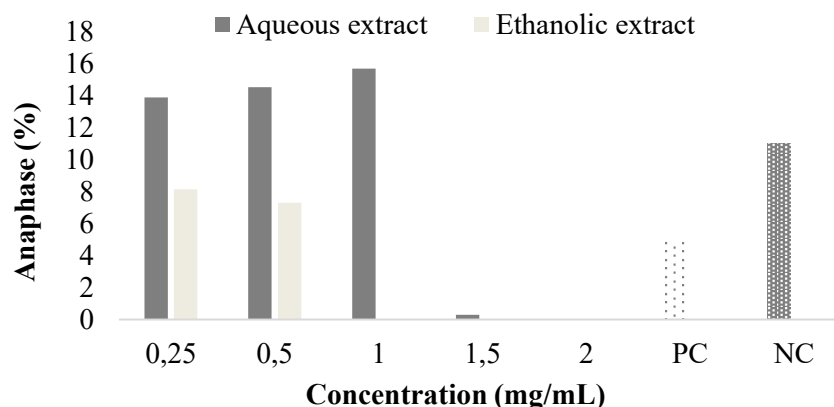


Figure 38. The effect of *A. galanga* extracts on anaphase in the root cells of *A. cepa*.

The *A. cepa* test results for the anaphase stage under treatment with *A. galanga* infusion reveal a variable and concentration-dependent response (Fig. 39).

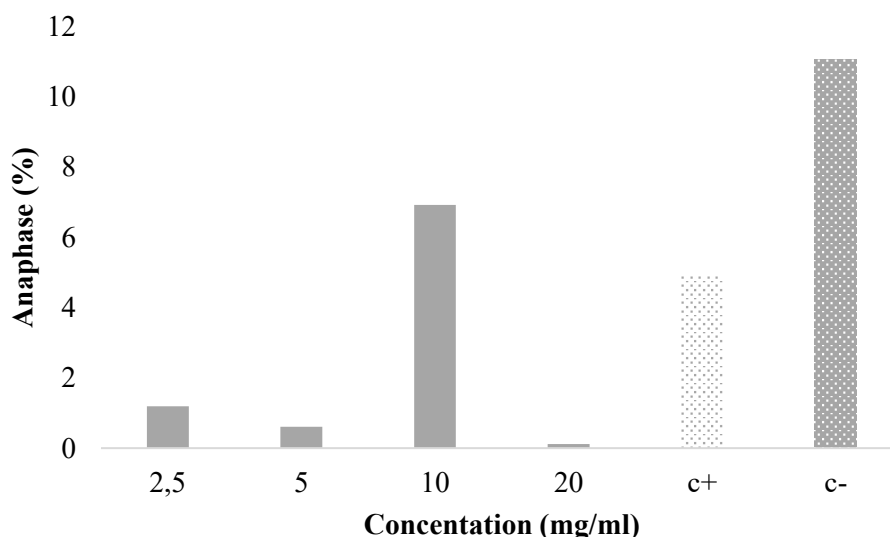


Figure 39. The effect of *A. galanga* infusion on anaphase in the root cells of *A. cepa*.

At 10 mg/mL, the number of anaphase cells peaked at 6.92, surpassing the positive control (4.91) but remaining lower than the negative control (11.05), suggesting a potential stimulatory effect at this dose. However, at 2.5 and 5 mg/mL, the values dropped significantly to 1.19 and 0.61, respectively. The lowest value was observed at 20 mg/mL (0.12).

• Telophase

Data analysis demonstrated that *A. galanga* extracts significantly influenced the telophase stage in *A. cepa* meristematic root cells (Fig. 40).

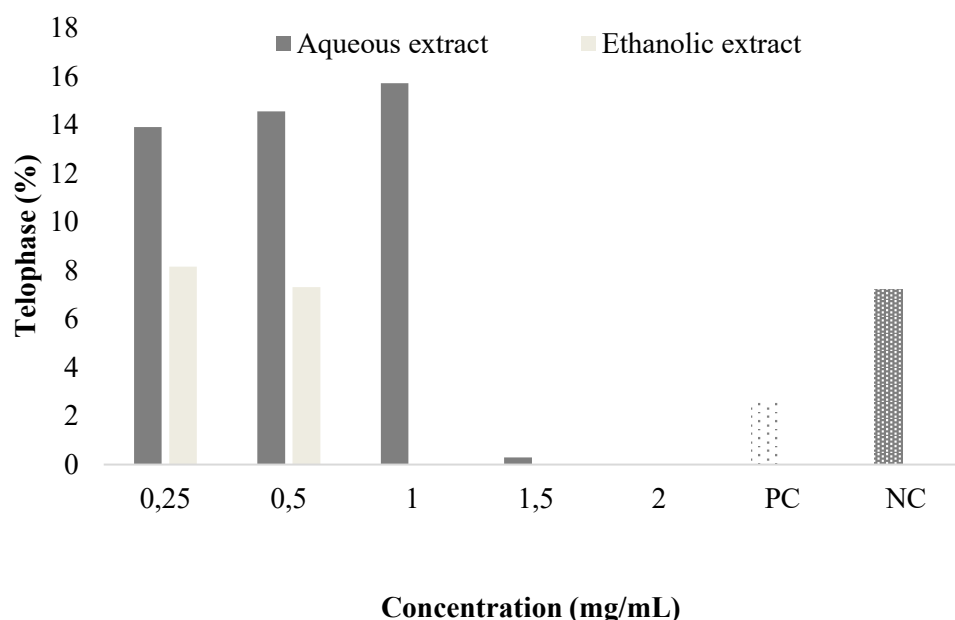


Figure 40. The effect of *A. galanga* extracts on telophase in the root cells of *A. cepa*.

For the aqueous extract, the number of telophase cells increased from 13.90% at 0.25 mg/mL to a peak of 15.71% at 1 mg/mL, before drastically decreasing to 0.29% at 1.5 mg/mL and reaching 0% at 2 mg/mL. In the ethanolic extract, the highest percentage of telophase cells was recorded at 0.25 mg/mL (8.15%), while no telophase cells were detected at 1 mg/mL and 2 mg/mL. These results highlight a concentration-dependent cytotoxic effect of the extracts, with higher doses severely reducing the frequency of telophase cells compared to the negative control (7.22%) and positive control (2.56%).

The telophase results from *A. cepa* root cells treated with *A. galanga* infusion reveal a dose-dependent response (Fig. 41).

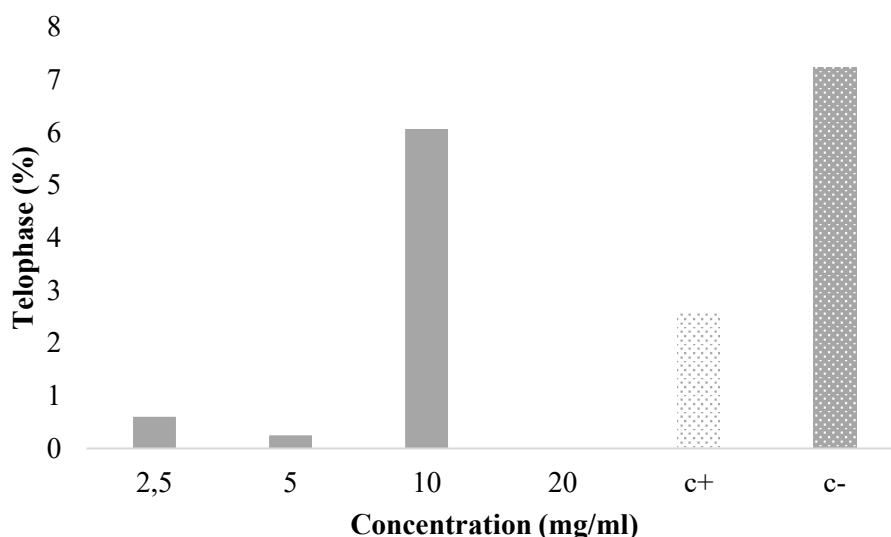


Figure 41. The effect of *A. galanga* infusion on anaphase in the root cells of *A. cepa*.

A notable increase in telophase cells was recorded at 10 mg/mL (6.06). However, at lower doses (2.5 and 5 mg/mL), the telophase frequencies were considerably reduced (0.6 and 0.25, respectively), and no telophase cells were observed at 20 mg/mL, reflecting a strong mitotic inhibition.

3.3. Evaluation of the genotoxicity

Microscopic examination of *Allium cepa* root tips treated with *A. galanga* extracts revealed a wide range of chromosomal abnormalities. Data analysis indicated that both aqueous and ethanolic extracts exerted considerable cytogenotoxic effects on the chromosomes. Notably, the aqueous extract induced a higher total number of aberrations (280) compared to the ethanolic extract (101). In both cases, the frequency of chromosomal abnormalities associated with anaphase-telophase stages, such as (disturbed anaphase, chromosomal laggards, bridges, and chromosomal stickiness) was lower than that of other mitotic anomalies, including (C-metaphase, polyploidy).

Discussion

Discussion

For centuries, medicinal plants have played a vital role in traditional healing practices across cultures. *Alpinia galanga*, a member of the Zingiberaceae family, is one such plant widely used in traditional medicine.

This study was conducted with three main objectives; first, to systematically document and preserve the valuable traditional knowledge surrounding the traditional use of *A. galanga* within Algerian communities; second, to scientifically analyze its chemical composition and identify its principal bioactive compounds; and third, to evaluate its biological safety by examining potential cytotoxic and genotoxic effects, using the *A. cepa* assay as a key investigative method.

Traditional medicine plays a key role in primary healthcare but requires clinical evaluation, practitioner training, and proper patient follow-up to ensure safety and effectiveness (Zaghwagh et al. 2015). Thus, the role of ethnopharmacology is to contribute to the development of modern therapeutic systems by building on ancestral knowledge and the traditional use of natural products to treat various diseases (Suntar 2019). Algerian people possess rich traditional knowledge regarding the use of aromatic and medicinal plants for treating and managing various health conditions (Djahafi et al. 2021). This valuable heritage, particularly related to the preparation and application of these plants, varies significantly from one region to another across the country (Taïbi et al., 2020).

A. galanga is traditionally recognized for its notable therapeutic properties and has long been used in folk medicine to treat various ailments. In addition to its curative uses, it is valued for its supportive roles in maintaining overall health. It helps regulate blood sugar and cholesterol levels, promotes digestion. Our findings reveal that *A. galanga* is predominantly used to treat respiratory ailments (33.5%), including colds, coughs, flu, and lung cleansing, that are consistent with its known antimicrobial and anti-inflammatory properties reported previously by Matsuda et al. (2003). It was also commonly applied for pain management (22%) such as headaches and menstrual pain, likely due to the presence of galangin and other flavonoids with analgesic effects (Mukhri et al., 2016). Cardiometabolic conditions accounted for 18.5% of its uses, particularly for hypotension, cholesterol control, and blood sugar regulation, aligning with its reported vasodilatory and hypoglycemic activities (Akhtar et al., 2020). This species is used also to treat digestive disorders (12.5%), reflecting its carminative and gastroprotective properties (Al-Yahya et al., 2018). Lastly, reproductive health uses (7.5%), such as postpartum

care and hormonal regulation, were less frequent and are not well documented in literature, suggesting a need for further research into its potential phytoestrogenic effects.

The wide range of these applications can be attributed to the richness of *A. galanga* in bioactive compounds such as flavonoids, tannins, and polyphenols (Eram et al., 2019). Its rhizome is particularly noted for its antifungal, antitumor, antimicrobial, anti-inflammatory, antidiabetic, antioxidant, and several other pharmacological properties (Chouni et Paul, 2017).

Decoction (45%) and infusion (40%) emerged as the most commonly used preparation methods, reflecting a preference for water-based extractions, which are globally recognized for efficiently isolating polar bioactive compounds such as polyphenols (Tiwari et al., 2011). Powder (5%) and honey mixtures (4%) were frequently employed, yet they allow for higher concentrations of active ingredients, with honey known to enhance bioavailability (Samarghandian et al., 2017). Rarely used methods, including topical applications and fumigation, indicate more specific or localized uses, likely targeting anti-inflammatory effects (Chouni & Paul, 2018). However, the restriction of use for pregnant women (120 mentions) may be linked to potential uterotonic or hormonal effects, as reported for other Zingiberaceae species (Ali et al., 2008).

The higher yield of ethanolic extract (14.8% vs. 13.8% aqueous) reflects ethanol's ability to solubilize both polar (phenolics) and mid-polar (flavonoids) compounds, as demonstrated by Alara et al. (2021) for Zingiberaceae species. The higher total phenolic content (TPC) observed in the hydroethanolic extract is likely due to ethanol's effective ability to break down plant cell walls and solubilize phenolic compounds (Tiwari et al., 2011). In contrast, the aqueous extract peaked at 1.5 mg/mL, possibly reflecting optimal solubility of certain water-soluble phenolics like gallic acid at moderate concentrations (Al-Yahya et al., 2018). Flavonoids such as quercetin, being moderately polar, are more efficiently extracted with ethanol (Sasidharan et al., 2018). For the infusion, the high total flavonoid content (TFC) at low concentrations may be attributed to the rapid release of surface-bound flavonoids, whereas prolonged heating or higher temperatures could lead to flavonoid degradation (Samarghandian et al., 2017). Tannins, are most effectively extracted at mid-range concentrations such as 0.5 mg/mL, likely due to an optimal balance between solubility and precipitation dynamics (Chouni and Paul, 2018). The infusion, meanwhile, showed a sharp increase in total tannin content (TTC), suggesting that heat enhances tannin release, an effect that aligns with traditional decoction methods used to extract tannin-rich compounds (Quave and Pieroni, 2015).

Despite their many benefits, the use of aromatic and medicinal plants can also pose risks. Some of their constituents may be toxic, leading to adverse effects that can harm human health. In severe cases, this can result in acute toxicity or even the death (Anywar et al., 2021).

Mitotic activity varied depending on the extract and concentration. The aqueous extract stimulated cell division at lower concentrations, with a maximum mitotic index of 27.06% at 1 mg/mL, possibly due to phenolic compounds acting as redox modulators (Gomes et al., 2022). However, at higher doses (2 mg/mL), mitotic activity dropped significantly to 13.41%, reflecting cytotoxic effects likely linked to oxidative stress induced by flavonoids and tannins (Prakash et al., 2021). The ethanolic extract exhibited a clear dose-dependent inhibition, with the mitotic index decreasing from 19.84% at 0.25 mg/mL to 11.87% at 1 mg/mL. This stronger cytotoxicity may be attributed to galangin, a dominant flavonoid in the extract known to interfere with microtubule polymerization (Silva et al., 2020). In metaphase analysis, the aqueous extract reached a peak frequency of 28.43% at 1 mg/mL before collapsing to 3.11% at 2 mg/mL, suggesting metaphase arrest due to spindle fiber anomalies. (Mao et al., 2019).

The infusion produced a moderate cytotoxic response, with the mitotic index peaking at 10 mg/mL (18.68%) and declining slightly at 20 mg/mL, indicating relatively lower toxicity compared to ethanolic and aqueous extracts. This reduced toxicity may be explained by the dilution of genotoxic compounds, as noted by Tungmunthum et al. (2018). The infusion also showed a strong dominance of prophase (99.77% at 20 mg/mL), suggesting a slowed yet ongoing cell cycle. Meanwhile, the limited presence of anaphase and telophase stages, with only 6.06% observed at 10 mg/mL, points to moderate interference with chromosomal segregation.

Polyploidy reflects a disruption in cytokinesis or instances of endoreduplication, phenomena commonly associated with flavonoids such as quercetin (Matsuda et al., 2003). The presence of C-metaphase indicates spindle apparatus dysfunction, potentially caused by tannins binding to spindle-associated proteins (Chouni and Paul, 2018). Chromosomal stickiness suggests DNA-protein crosslinking, likely triggered by oxidative stress induced by phenolic compounds (Turkoglu, 2007). Chromosome laggards at anaphase may be due to the failure of the chromosomes or acentric chromosome fragments to move to either of the pole and may result in micronucleate cells. Interestingly, the observed inverse dose-response—fewer chromosomal aberrations at higher concentrations, may be explained by increased cell death (via apoptosis or necrosis), which limits the division of genetically damaged cells (Rank and Nielsen, 1993).

For instance, *in vitro* studies on human cancer cell lines have shown that *A. galanga* ethanolic extracts possess significant cytotoxic activity, attributed to bioactive compounds like galangin and phenolic acids (Matsuda et al., 2003; Srividya et al., 2010). Similarly, our *A. cepa* tests confirmed that ethanolic extracts induce higher mitotic inhibition and chromosomal aberrations (polyploidy, c-metaphase) compared to aqueous extracts, likely due to their greater concentration of flavonoids and tannins.

Conclusion & perspectives

Conclusion

Many medicinal plants are traditionally used to treat a variety of health conditions. *A. galanga*, widely employed in Algerian folk medicine, is known for its benefits in managing respiratory, digestive, and reproductive ailments. However, like any other plant, *A. galanga* may also present potential toxic effects.

This study presents an in-depth evaluation of the ethnopharmacological potential, phytochemical profile, and cyto-genotoxic effects of *A. galanga* through the *A. cepa* bioassay, using aqueous, hydroethanolic, and infusion extracts.

Ethnopharmacological study reveals that *A. galanga* is mainly used for respiratory problems like colds and coughs. It's also used for pain relief, and cardiometabolic conditions. Some rare uses include fertility, cancer, and improving memory, while others simply use it in coffee for flavor.

Phytochemical analyses demonstrate that the hydroethanolic extract exhibited higher contents of polyphenols, flavonoids, and tannins compared to the aqueous extract, demonstrating the superior solvent efficiency of ethanol. Interestingly, the infusion also showed a considerable presence of these bioactive compounds, especially at higher concentrations

Cytogenotoxic shows that both aqueous and hydroethanolic extracts showed dose-dependent effects on root number and length, with stimulation at lower doses and inhibition at higher concentrations. The infusion, however, showed moderate stimulation of cell division, with a peak mitotic index at 10 mg/mL.

The hydroethanolic extract showed stronger toxicity, especially at concentrations above 1 mg/mL, leading to mitotic arrest and chromosomal abnormalities. The infusion exhibited moderate cytotoxicity and the lowest genotoxic impact, indicating a potentially safer traditional preparation method.

Given these findings, precaution is advised when using *A. galanga* for therapeutic purposes, particularly regarding dosage and preparation method. Further toxicological and pharmacological studies are necessary to better understand the safety and efficacy profile of this medicinal plant.

References