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MODELAGEM DA LEUCEMIA MIELOIDE AGUDA COM CÉLULAS THP-1: EXPLORANDO O POTENCIAL ANTILEUCÊMICO DE DIFERENTES EXTRATOS DE PANAX QUINQUEFOLIUM

MODELING ACUTE MYELOID LEUKEMIA WITH THP-1 CELLS: EXPLORING THE ANTILEUKEMIC POTENTIAL OF DIFFERENT PANAX QUINQUEFOLIUM EXTRACT

نموذج ابيضاض الدم النقوي الحاد باستخدام خلايا THP-1: استكشاف الإمكانات المضادة لسرطان الدم لمستخلصات مختلفة من PANAX QUINQUEFOLIUM

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RESUMO

Introdução: A leucemia mieloide aguda é uma malignidade hematológica caracterizada pela proliferação descontrolada de blastos mieloides imaturos, resultando em prognóstico desfavorável e opções terapêuticas limitadas. A linha celular THP-1, derivada de um paciente com leucemia monocítica aguda, serve como modelo in vitro confiável para monócitos e macrófagos humanos. Produtos naturais, como Panax quinquefolium (ginseng americano), contêm compostos bioativos com propriedades antioxidantes, anti-inflamatórias e anticancerígenas, oferecendo alternativas terapêuticas mais seguras. **Objetivo**: Avaliar o potencial antileucêmico de quatro extratos diferentes de raízes de Panax quinquefolium em células THP-1. **Métodos**: As células THP-1 foram tratadas com extratos aquosos, hexano, etanol e clorofórmio em concentrações de 6,25 a 200 µg/ml. A citotoxicidade foi avaliada pelo ensaio MTT, e seções histológicas foram analisadas para densidade e morfologia celular. **Resultados**: Todos os extratos inibiram a proliferação das células de forma dependente da dose, com diferenças significativas entre os tratamentos ($p < 0,001$). O extrato de clorofórmio foi o mais eficaz, reduzindo o crescimento em 88,11 ± 3,14% a 200 µg/ml, seguido por etanol, hexano e aquoso. A histologia confirmou os achados: células controle formaram aglomerados densos; o extrato aquoso reduziu modestamente a densidade; hexano e etanol causaram reduções maiores; o clorofórmio resultou no menor número de células viáveis. **Discussão**: Os resultados indicam que os extratos de Panax quinquefolium possuem atividade antileucêmica dependente da dose, com o clorofórmio sendo o mais potente. As diferenças entre os extratos provavelmente refletem variações na polaridade e solubilidade dos compostos bioativos. Produtos naturais podem oferecer alternativas mais seguras que a quimioterapia convencional, embora estudos adicionais sejam necessários para esclarecer os mecanismos moleculares e a eficácia em organismos vivos. **Conclusão**: O extrato de clorofórmio de Panax quinquefolium suprime efetivamente a proliferação de células THP-1, demonstrando forte atividade antileucêmica e potencial como agente terapêutico seguro e promissor.

Palavras-chave: *Extrato de Panax quinquefolium, Inibição da leucemia mieloide aguda, Linha celular THP-1, Antileucêmico.*

ABSTRACT

Background: Acute Myeloid Leukemia (AML) is a hematological malignancy characterized by uncontrolled proliferation of immature myeloid blasts, leading to poor prognosis and limited treatment options. The THP-1 cell line, derived from a patient with acute monocytic leukemia, serves as a reliable in vitro model for human monocytes and macrophages. Natural products, such as Panax quinquefolium (American ginseng), contain

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bioactive compounds with antioxidant, anti-inflammatory, and anticancer properties, offering safer alternatives for AML therapy. **Aim**: To evaluate the antileukemic potential of four P. quinquefolium root extracts on THP-1 cells. **Methods**: THP-1 cells were treated with aqueous, hexane, ethanol, and chloroform extracts at 6.25–200 µg/ml. Cytotoxicity was assessed via the MTT assay, and histological sections were analyzed for cell density and morphology. **Results**: All extracts inhibited THP-1 proliferation in a dose-dependent manner, with significant differences among treatments ($p < 0.001$). The chloroform extract was the most effective, reducing growth by 88.11 ± 3.14% at 200 µg/mL, followed by the ethanol, hexane, and aqueous extracts. Histology confirmed: control cells formed dense clusters; aqueous extract modestly reduced density; hexane and ethanol caused further reductions; chloroform yielded the fewest viable cells. **Discussion**: These findings indicate potent dose-dependent antileukemic activity of P. quinquefolium, particularly its chloroform extract. The variation among extracts likely reflects differences in polarity and solubility of bioactive compounds. Natural products may offer safer alternatives to conventional chemotherapy; however, further studies are required to elucidate their molecular mechanisms and in vivo efficacy. **Conclusion**: The chloroform extract of Panax quinquefolium effectively suppresses THP-1 proliferation, demonstrating strong antileukemic activity and supporting its potential as a safe and promising therapeutic candidate for AML.

Keywords: *Panax quinquefolium extract, AML inhibition, THP-1 cell line, and Antileukemic.*

الخلفية: ابيضاض الدم النقوي الحاد (AML) هو سرطان دموي يتميز بالتكاثر غير المنضبط للأورمات النخاعية غير الناضجة، مما يؤدي إلى سوء الإنذار وقلة خيارات العلاج المتاحة. يُعد خط الخلايا THP-1 المشتق من مريض مصاب بابيضاض الدم النقوي الوحيدي نموذجاً موثوقاً لدراسة الوحيدات والبلاعم البشرية في المختبر. تحتوي المنتجات الطبيعية، مثل *Panax quinquefolium* (الجنسنغ الأمريكي)، على مركبات فعالة بخصائص مضادة للأكسدة والالتهاب والسرطان، مما يوفر بدائل علاجية أكثر أماناً لعلاج AML. **الهدف:** تقييم النشاط المضاد لسرطان الدم لأربعة مستخلصات مختلفة من جذور *P. quinquefolium* على خلايا THP-1. **طرائق العمل:** تمت معالجة خلايا THP-1 بمستخلصات مائية، هكسان، إيثانول، وكلوروفورم بتركيزات تتراوح بين 6.25–200 مايكروغرام/ملليتر. تم تقييم التأثيرات السامة للخلايا باستخدام اختبار MTT ، كما أُجريت مقاطع نسيجية لدراسة كثافة الخلايا ومورفولوجيا الخلايا. **النتائج:** ثبطت جميع المستخلصات تكاثر خلايا THP-1 بطريقة معتمدة على الجرعة، مع اختلافات معنوية بين المعالجات ($p < 0.001$). أظهر المستخلص الكلوروفورمي أقوى تأثير، حيث خفّض نمو الخلايا بنسبة 88.11 ± 3.14% عند 200 مايكروغرام/ملليتر، يليه مستخلص الإيثانول، الهكسان، والمائي. دعمت الفحوص النسيجية هذه النتائج حيث شكلت خلايا السيطرة تجمعات كثيفة، وقلّلت المستخلصات المائية والهكسانية والإيثانولية الكثافة تدريجياً، بينما أنتج الكلوروفورم أقل عدد من الخلايا الحية. **المناقشة:** تشير النتائج إلى أن مستخلصات *P. quinquefolium* تمتلك نشاطاً مضاداً للابيضاض بطريقة معتمدة على الجرعة، مع تفوق المستخلص الكلوروفورمي. تعكس الاختلافات بين المستخلصات فروقاً في القطبية وقابلية ذوبان المركبات الفعالة. قد توفر المنتجات الطبيعية بدائل أكثر أماناً مقارنة بالعلاج الكيميائي التقليدي، مع ضرورة إجراء دراسات إضافية لتوضيح الآليات الجزيئية وفعالية المستخلصات في الجسم الحي. **الاستنتاج**: يثبط المستخلص الكلوروفورمي لجذور *P. quinquefolium* تكاثر خلايا THP-1 بفعالية، مظهراً نشاطاً مضاداً قوياً للابيضاض، مما يدعم إمكاناته كعامل علاجي واعد وآمن لمرضى AML.

الكلمات المفتاحية: مستخلص الجينسنغ الأمريكي، تثبيط ابيضاض الدم النقوي الحاد، خط خلايا ***THP-1***، مضاد للابيضاض

1. INTRODUCTION:

Acute Myeloid Leukemia (AML) is a hematological malignancy characterized by the uncontrolled proliferation of immature myeloid leukemic blasts (Shimony *et al.,* 2025). AML represents a highly heterogeneous disease, involving chromosomal abnormalities, molecular genetics, biochemical alterations, and mutations (Hakon *et al.,* 2023). It accounts for approximately 33% of all hematological cancers and is associated with a 5-year survival rate of only 24% (Gyi *et al.,* 2025). Globally, AML ranks among the leading causes of cancer-related mortality and was the 13th most frequently diagnosed malignancy in 2022 (Bray *et al*., 2022).

The THP-1 cell line, derived from a patient with acute monocytic leukemia at the age of one (Jo *et a*l., 2024), serves as a reliable in vitro model of monocytes and macrophages. THP-1 cells are particularly useful for mimicking the tumor microenvironment due to their similarity with primary monocytes and their capacity for macrophage-like differentiation. Conventional chemotherapeutic drugs used in AML treatment are often associated with severe side effects, highlighting the need for safer and effective alternatives (James *et al*., 2023).

Panax quinquefolium (American ginseng) is a medicinal plant originally from North America and cultivated in regions such as Ontario. Cultivated ginseng in China, imported from North America, contains fewer lateral roots and lower levels of active compounds such as ginsenosides compared to wild American ginseng roots (Fang *et al*., 2023). The therapeutic properties of ginseng are primarily attributed to ginsenosides, which exhibit anti-apoptotic, antioxidant, and anti-inflammatory effects. The root is rich in saponins, including bioactive ginsenosides, which have demonstrated pharmacological and therapeutic benefits (Li *et al*., 2023). Recent studies have highlighted the potential of ginseng in cancer therapy, cognitive enhancement, and cardiovascular protection (Zhang *et al.,* 2024).

This study aimed to investigate the anticancer

potential of different P. quinquefolium root extracts (aqueous, hexane, ethanol, and chloroform) on THP-1 leukemia cells, focusing on dose-dependent growth inhibition and histological alterations, to evaluate their anti-leukemia effects.

2. MATERIALS AND METHODS:

2.1. Materials

Plant Material

Panax quinquefolium root powder (source and authentication details as Figure 1.

Figure 1 . Panax quinquefolium root powder

Extraction Solvents

- Absolute ethanol (analytical grade)
- Chloroform (HPLC grade)
- n-Hexane (analytical grade)
- Distilled water (sterile)

Cell Culture Materials

- THP-1 human acute monocytic leukemia cell line (ATCC® TIB-202™)
- RPMI-1640 medium (Gibco, Life Technologies)
- Fetal Bovine Serum (FBS) (Gibco, Life Technologies)
- Penicillin-Streptomycin solution (10,000 U/mL) (Gibco, Life Technologies)
- L-Glutamine (200 mM) (Gibco, Life Technologies)
- Phosphate Buffered Saline (PBS) (pH 7.4)
- Trypan blue solution (0.4%)

Cell Viability Assay Materials

- 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) powder
- Dimethyl sulfoxide (DMSO) (cell culture grade)
- 96-well flat-bottom microplates (sterile)
- 24-well cell culture plates (sterile)

Histological Staining

- Crystal violet solution (0.5% w/v)
- Methanol (for fixation)

Laboratory Equipment

- Ultrasonic bath (frequency: 40 kHz)
- CO_2 incubator (37 °C, 5% CO_2)
- Class II biological safety cabinet
- Inverted microscope with phase contrast
- Microplate reader (capable of reading at 492 nm)
- Centrifuge (benchtop)
- Analytical balance (0.1 mg precision)
- Refrigerator (4 °C) and freezer (-20 °C)
- Drying oven (40 °C)
- Autoclave for sterilization

Filtration and Purification

- Whatman filter paper No. 1
- 0.22 µm sterile syringe filters
- Sterile syringes (various sizes)

HPLC Analysis Materials

- High-Performance Liquid Chromatography (HPLC) system
- C18 reverse-phase column (specifications should be provided)
- HPLC-grade solvents (acetonitrile, methanol, water)
- Standard reference compounds (if available)

General Laboratory Consumables

- Micropipettes (10-1000 µL range)
- Sterile pipette tips (filtered)
- Serological pipettes (5, 10, 25 mL)
- Microcentrifuge tubes (1.5 mL, 2.0 mL)
- Cell culture flasks (T25, T75)
- Glass vials for extract storage
- Parafilm for sealing
- Disposable gloves (nitrile)

- Face masks and laboratory coats

2.2. Methods

2.2.1. Ginseng Extracts Preparation

For the preparation of ginseng extracts, four distinct solvents —ethanol, chloroform, hexane, and distilled water —were employed. A precise volume of 100 mL of each solvent was added to 20 grams of powdered ginseng root. Subsequently, the traditional extraction method was augmented by ultrasonic-assisted extraction (UAE), a technique validated by Yusoff *et al.* (2022). This approach facilitates the penetration of the solvent into cellular structures, where acoustic cavitation-induced bubbles effectively disrupt cell walls, thereby promoting the release of active compounds, notably ginsenosides, and other constituents. The extraction mixtures were then incubated for 24 hours at ambient temperature. Following incubation, the mixtures were meticulously filtered using Whatman filter paper to obtain the filtrate, which was subsequently dried in an oven at 40 °C. The resulting dry extracts were stored under refrigerated conditions until further use, consistent with the methodology described by (Tian *et al*., 2013).

2.2.2. Cytotoxicity Assay

To determine the cytotoxic potential of each of the four extracts, the MTT assay was performed using 96-well plates, following established protocols (Al-Ziaydi *et al.,* 2020). Cells were seeded at a density of 1×10^4 cells/well and incubated for 24 hours to achieve a confluent monolayer. Human monocytic THP-1 cells, serving as an in vitro model for primary human monocytes, were treated with each P. ginseng root extract. Cell viability was assessed at 24 and 48 hours post-treatment. This involved the removal of the culture medium, followed by the addition of 28 µL of a 2 mg/mL MTT solution. The cells were then incubated for 2.5 hours at 37 °C. Following the removal of the MTT solution, the formazan crystals remaining in the wells were solubilized by adding 130 µL of Dimethyl Sulfoxide (DMSO), followed by a 15-minute incubation at 37 °C with gentle shaking. Absorbance was quantified at 492 nm using a microplate reader. All assays were conducted in triplicate. The inhibition rate of cell growth (cytotoxicity percentage) was calculated using Equation 1

$$\text{Inhibition rate (cytotoxicity)} = (A - B) / A \times 100 \quad \text{(Eq. 1)}$$

Where: A represents the optical density of the control, and B represents the optical density of the samples (Ibrahim *et al*., 2021).

For morphological assessment, cells were seeded into 24-well microtiter plates at a density of 1×10^5 cells/mL and incubated for 24 hours at 37 °C. Subsequently, cells were exposed to each type of ginseng root extract for 24 hours. Post-exposure, the plates were stained with crystal violet and incubated at 37 °C for 10-15 minutes. The stain was then carefully rinsed with tap water until complete removal of the dye was achieved (Jabir *et al*., 2022).

THP-1 cells were maintained in RPMI-1640 supplemented with 10% Fetal bovine serum, 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were passaged using Trypsin-EDTA, reseeded at 80% confluence twice a week, and incubated at 37 °

2.3. Statistical Analysis

All experiments were conducted using a completely randomized design with appropriate controls. Each treatment condition was performed in technical triplicate within each experiment, and experiments were independently repeated three times (n = 3 independent experiments) to ensure biological reproducibility. Data from individual experiments were pooled only after confirming consistency across replicates.

Data are presented as mean ± standard deviation (SD) calculated from all measurements across the three independent experiments. Prior to statistical analysis, data normality was assessed using the Shapiro-Wilk test, and homogeneity of variances was evaluated using Levene's test. When assumptions were violated, appropriate data transformations were applied.

Statistical analysis was performed using SPSS version 27.0 (IBM Corp., Armonk, NY, USA). For cytotoxicity data comparison across different extract types and concentrations, one-way analysis of variance (ANOVA) was employed, followed by Tukey's honestly significant difference (HSD) post-hoc test for pairwise multiple comparisons between treatment groups. Two-way ANOVA was used to analyze the interaction effects between extract type and concentration.

IC_{50} values were determined by fitting the dose–response data to a four-parameter logistic (4PL) model. Curve fitting and statistical analysis

were performed using spss 27. The quality of the fit was assessed by examining goodness-of-fit metrics, including the coefficient of determination (R^2) and the distribution of residuals was calculated using Equation 2

Response = Bottom + (Top - Bottom) / (1 + 10^((logIC_50 – LogConc.) * Hill Slope)

(Eq. 2)

The estimated IC50 (half-maximal inhibitory concentration) values for the different extracts approxmatly are as follows:

Chloroform: Between 25 and 35 µg/ml.
Ethanol: Between 30 and 40 µg/ml.
Hexane: Between 35 and 50 µg/ml.
Water: Between 40 and 50 µg/ml.

Statistical significance was set at $\alpha = 0.05$ for all analyses. P-values were adjusted for multiple comparisons using the Bonferroni correction when appropriate. All statistical analyses and graphical representations were generated using SPSS software. Results with $p < 0.05$ were considered statistically significant, with $p < 0.01$ and $p < 0.001$ indicating higher levels of significance as specified in the results.

3. RESULTS AND DISCUSSION:

3.1. Results

3.1.1. Cytotoxicity of Panax Ginseng Extracts from Different Solvents on THP-1 Cells at Various Concentrations

The antileukemic effects of four Panax ginseng extracts, Aqueous (Water), Hexane, Ethanol, and Chloroform were evaluated against THP-1 cells in vitro. All extracts exhibited cytotoxic effects with varying degrees of tumor inhibition. The results in Table 1 revealed mean activity values across six concentrations (6.25–200 µg/ml). At 6.25 µg/ml, chloroform had the highest activity (12.67 ± 0.90), followed by Ethanol (9.62 ± 0.84), Hexane (8.30 ± 1.03), and Water (6.59 ± 0.91). This pattern persisted at 200 µg/ml, with chloroform at the highest level (88.11 ± 3.14), followed by ethanol (84.15 ± 3.39), hexane (80.43 ± 3.64), and water at the lowest level (77.35 ± 2.65l). Ethanol consistently ranked second, hexane was an intermediate, and water ranked lowest across all concentrations. Compound levels increased significantly with dose for all extracts, indicating a dose-dependent effect Figure 2. Differences among extracts were statistically significant ($p < 0.001$).

3.1.1. Histological examination

The histological examination of four types of solvent ginseng extract provides valuable insight into the morphological changes induced by cancer cells following exposure to ginseng extract active compounds, as shown in Figure 3 (A-E). In the current study, the human acute monocytic leukemia (THP-1) cells were treated with four types of different solvent extracts of Panax ginseng, including water, ethanol, and chloroform. To evaluate their cytotoxicity potential. Crystal violet staining was used to visualize cell morphology and density, allowing for a direct comparison between the treated groups and the control group. Suppression effects on cell viability and proliferation, as compared to the degree of reduction across treatments, this histological analysis highlights the relative efficacy of each ginseng extract in inhibiting THP-1 cell growth.

3.2. Discussion

3.2.1. The Effect of Bioactive Compounds of Ginseng – Aqueous Extract on THP-1 Cell Line

The aqueous extract of *Panax ginseng* root is particularly rich in polar bioactive compounds, including ginsenosides (Rg1, Re, Rd), immunomodulatory polysaccharides (panaxans), and saponins that contribute to its wide pharmacological properties (Srisuk *et al*., 2024; Lertchanyaporn *et al*., 2024). Ginseng phytochemicals are broadly classified into polysaccharides, polyynes, flavonoids, and volatile oils (Jia & Zhao, 2009). Importantly, ginsenosides and water-soluble polysaccharides exhibit immunomodulatory and anti-proliferative effects (Poudyal *et al*., 2012).

The aqueous extract is characterized by high polarity and a composition enriched in ginsenosides (Rg1, Re, Rb1), phenolics, and small peptides, which collectively demonstrate strong cytotoxic and anti-proliferative activity against THP-1 leukemia cells (Kim *et al*., 1998; Shim *et al*., 2007). This finding aligns with previous studies that ginsenosides Rg3 and Rh2 induce apoptosis through mitochondrial ROS generation (Xia *et al.,* 2017; Dana *et al*., 2024).

Mechanistically, ginsenoside Rh2 (GRh2) induces G1-phase cell cycle arrest (Chen *et al*., 2016; Huang *et al*., 2016), acts as a histone deacetylase (HDAC) inhibitor, and regulates

apoptosis-related proteins (Bcl-2/Bax) (Liu *et al*., 2015). Likewise, ginsenosides Rg1 and Rb1 promote apoptosis through the activation of caspase-3, mitochondrial dysfunction, and cytochrome c release (Li *et al.,* 2014; Zhou *et al*., 2019). Polysaccharide fractions further enhance macrophage activity and cytokine secretion (IL-1β, IL-6, TNF-α), strengthening innate immunity (Choi *et al*., 2023).

3.2.2. The Effect of Bioactive Compounds of Ginseng – Hexane Extract on THP-1 Cell Line

Among the extracts compared in Table 1, the hexane fraction exhibited remarkable cytotoxic effects on THP-1 cells, primarily due to its enrichment in ginsenoside Rg2. This bioactive compound induces apoptosis, inhibits angiogenesis, and suppresses metastasis (Zhang et al., 2021). Notably, ginsenoside Rh2 blocks epithelial–mesenchymal transition (EMT), a hallmark of metastasis (Tied *et al.,* 2019, Kim *et al.,* 2017).

Ginsenoside Rg2 also exhibits immunomodulatory, antioxidant, and anti-inflammatory effects (Qian *et al*., 2019; *Huang et al*., 2019). These are mediated via mitochondrial apoptosis, involving caspase-3 activation, PARP cleavage, and modulation of the Bax/Bcl-2 ratio (Lee *et al*., 2020; Kim *et al.,* 2021). NF-κB suppression also contributes to the inhibition of anti-survival genes (Jang *et al.,* 2023).

The current results in Table 1 demonstrated that the hexane extract caused a cytotoxic effect of 46.30 ± 27.24 against THP-1 cells. This supports its potential as an anticancer agent and, given the lack of prior leukemia-specific reports, underscores the novelty of the present study (Cui *et al.,* 2010).

3.2.3. The Effect of Bioactive Compounds of Ginseng – Ethanolic Extract on THP-1 Cell Line

As summarized in Table 1, the ethanoic extract (70%) of Panax ginseng demonstrated the highest tumor cell killing ratio (84.15 ± 3.39), surpassing aqueous and hexane extracts. This potency is attributed to its higher concentration of ginsenosides (Rg3, Rg5, Rg6), phenolics, and flavonoids (Walia *et al.,* 2023). These compounds exert strong antioxidant activity, reducing ROS generation and protecting cellular integrity. The apoptotic mechanisms involve both the intrinsic (mitochondrial) and extrinsic (death receptor) pathways (Yang *et al.,* 2023; Moyer, 2025). Our findings align with reports highlighting ethanol extracts as the richest in antioxidant and cytotoxic compounds compared with other solvent fractions (Hasan *et al.,* 2025).

3.2.4. The Effect of Bioactive Compounds of Ginseng – Chloroform Extract on THP-1 Cell Line

The chloroform extract of Panax ginseng demonstrated the strongest cytotoxic activity among all tested extracts. This fraction is enriched with β-phytosterols and fatty acids, which modulate membrane dynamics, promote apoptosis, and inhibit metastasis (Qi *et al.,* 2011).

Our findings confirm earlier reports that β-phytosterol is a natural anticancer compound with apoptosis-inducing and cancer risk–reducing effects (Dąbrowska *et al.,* 2023). HPLC chromatographic profiling (Table 2) revealed 16 peaks, with four major active compounds (peaks 4, 6, 7, and 11) (Figure 4) contributing significantly to cytotoxicity. These bioactive constituents include fatty acids with anti-proliferative, anti-inflammatory, and anticancer properties (Christmann *et al.,* 2022; Budi *et al.,* 2022).

Thus, chloroform-extracted ginseng holds great promise as a potent anticancer candidate due to its unique phytochemical composition, which differs from that of more polar extracts.

4. **Conclusions**:

This study evaluated the cytotoxic potential of four different Panax quinquefolium root extracts (aqueous, hexane, ethanol, and chloroform) against THP-1 acute myeloid leukemia cells using MTT viability assays and morphological analysis. All tested extracts demonstrated dose-dependent inhibition of THP-1 cell proliferation across concentrations ranging from 6.25 to 200 µg/ml, with statistically significant differences observed between treatments ($p < 0.001$). The chloroform extract exhibited the highest cytotoxic activity, achieving 88.11 ± 3.14% growth inhibition at the maximum tested concentration, followed by the ethanol extract (84.15 ± 3.39%), the hexane extract (80.43 ± 3.64%), and the aqueous extract (77.35 ± 2.65%). Histological observations using crystal violet staining corroborated the quantitative findings, showing progressive reduction in cell density and viability corresponding to the cytotoxic potency of each extract. HPLC analysis of the chloroform extract identified four major bioactive peaks, including hexadecanoic acid and methyl13-methyltetradecanoate, which may contribute to the

observed cytotoxic effects.

These findings suggest that Panax quinquefolium extracts, particularly the chloroform fraction, possess significant anti-proliferative activity against THP-1 cells and warrant further investigation. Future studies should include mechanistic analyses to elucidate the specific pathways involved in the observed cytotoxicity, assess selectivity toward cancer versus normal cells, and evaluate synergistic effects with conventional chemotherapeutic agents. The results provide preliminary evidence supporting the potential therapeutic value of P. quinquefolium extracts in the treatment of acute myeloid leukemia. However, extensive preclinical and clinical validation will be necessary before clinical application.

5. DECLARATIONS

5.1. Study Limitations

This study was conducted in vitro using the THP-1 cell line, which may not fully represent in vivo effects. Whole Panax quinquefolium extracts were used without identifying specific bioactive compounds, and molecular mechanisms were not investigated. Only short-term exposures were assessed, and other concentrations may yield different results. These limitations underscore the need for further in vivo studies and mechanistic research to confirm and expand upon these findings.

5.2. Acknowledgements

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5.3. Funding source

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5.4. Competing Interests

The authors declare that they have no competing interests or conflicts of interest that could have influenced the work presented in this manuscript. This includes, but is not limited to, financial relationships, personal affiliations, intellectual property considerations, or other potential sources of bias. All authors have reviewed and approved this declaration, ensuring transparency and maintaining scientific integrity in the reporting of this research.

5.6. Use of AI

The manuscript was written in English with careful attention to clarity and precision. AI tools were employed solely to enhance language quality and translate the abstract into Portuguese. All scientific content, terminology, and methodology were thoroughly reviewed and validated by the authors, who remain fully responsible for the accuracy and integrity of the work.

6. HUMAN AND ANIMAL-RELATED STUDIES

6.1. Ethical Approval

This study was conducted using a

commercially available/preserved cancer cell line for research purposes only, provided by the Phi Nano Sciences Center (PNSC), without involving human participants or experimental animals. Therefore, no approval from human or animal ethics committees was required. Nevertheless, the research strictly adhered to the guidelines outlined in the Research Ethics Book, Faculty of Science, University of Kufa, dated June 29, 2025, and all biosafety procedures (Biosafety Guidelines) were carefully followed during cell culture handling and laboratory work.

6.2. Informed Consent

Not applicable. This study was conducted exclusively using a commercially available/preserved cancer cell line, without involving human participants or experimental animals; therefore, informed consent was not required.

7. REFERENCES:

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Table 1: *Cytotoxicity of Panax Ginseng Extracts from Different Solvents on THP-1 Cells at Various Concentrations*

Concentration	**(Mean ± SD)**				
(µg/ml)	**Ethanol**	**Chloroform**	**Hexane**	**Water**	**Total**
GA: 6.25	9.62 ± 0.84 b	12.67 ± 0.90 a	8.30 ± 1.03 bc	6.59 ± 0.91 c	9.29 ± 2.45
GB: 12.5	22.35 ± 1.50 b	27.37 ± 1.57 a	18.97 ± 1.50 bc	16.81 ± 2.58 c	21.37 ± 4.45
GC: 25	45.62 ± 2.59 ab	49.20 ± 2.96 a	40.45 ± 2.76 bc	38.53 ± 2.70 c	43.45 ± 4.99
GD: 50	62.49 ± 2.81 ab	65.95 ± 4.23 a	57.70 ± 2.52 bc	54.05 ± 3.34 c	60.05 ± 5.50
GE: 100	75.96 ± 3.83 b	78.73 ± 3.64 a	71.97 ± 2.84 bc	67.70 ± 4.72 c	73.59 ± 5.43
GF: 200	84.15 ± 3.39 ab	88.11 ± 3.14 a	80.43 ± 3.64 bc	77.35 ± 2.65 c	82.51 ± 5.03
Total	50.03 ± 27.99	53.67 ± 27.84	46.30 ± 27.24	43.51 ± 26.50	48.38 ± 27.09
p-value			<0.001		

Different small letters expressed significant differences between the extract groups at p-value <0.05 by ANOVA with Tukey's test

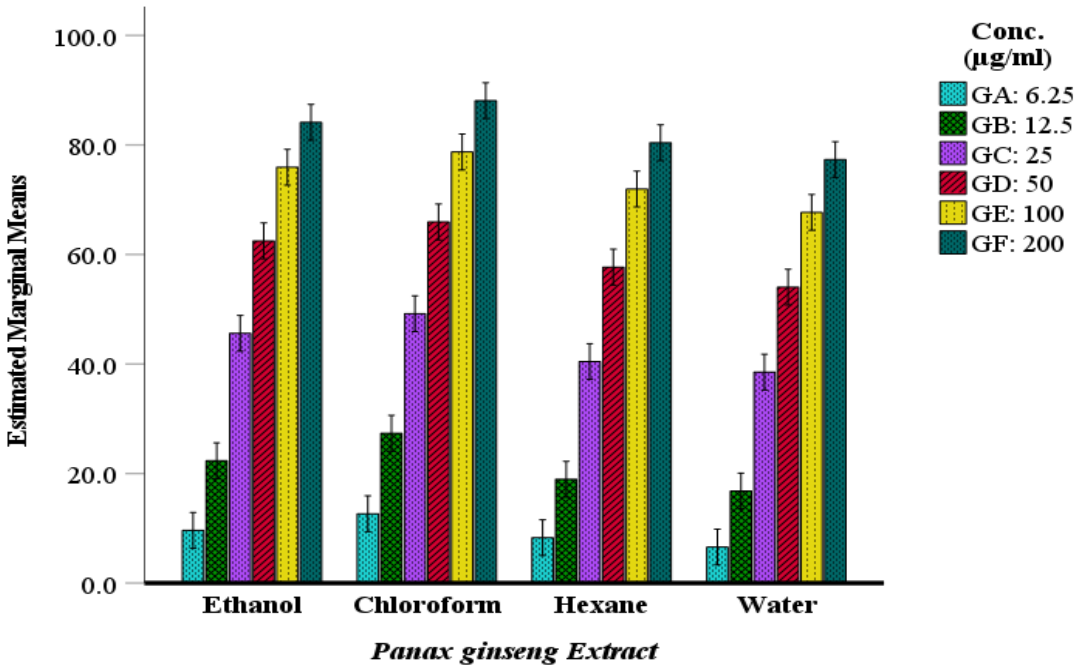

Figure 2: *Inhibitory Potential of Different Panax Ginseng Extracts on THP-1 Cells*

Table 2*: Peaks identified in the HPLC chromatographic profile of the chloroform extract of ginseng, highlighting four major peaks—Peak 4, Peak 6, Peak 7, and Peak 11*

Peak	R .time	Area %	Height %	Comp Name	Formula	Molecule Weight
4	17.049	21.35	19.35	Hexadecanoic acid	C17H34O2	270
6	2.328	0.92	0.82	TetrahydropyranZ-10-dodecenoate	C17H30O3	282
7	17.041	34.86	33.33	Methyl13-methyltetradecanoate	C16H32O2	256
11	2.016	0.17	1.02	2-Formylhistamine	C6H9N3O	139

Table 2 shows the four major peaks (>5% area) identified in the HPLC analysis. Complete chromatographic data is available in Supplementary Material.

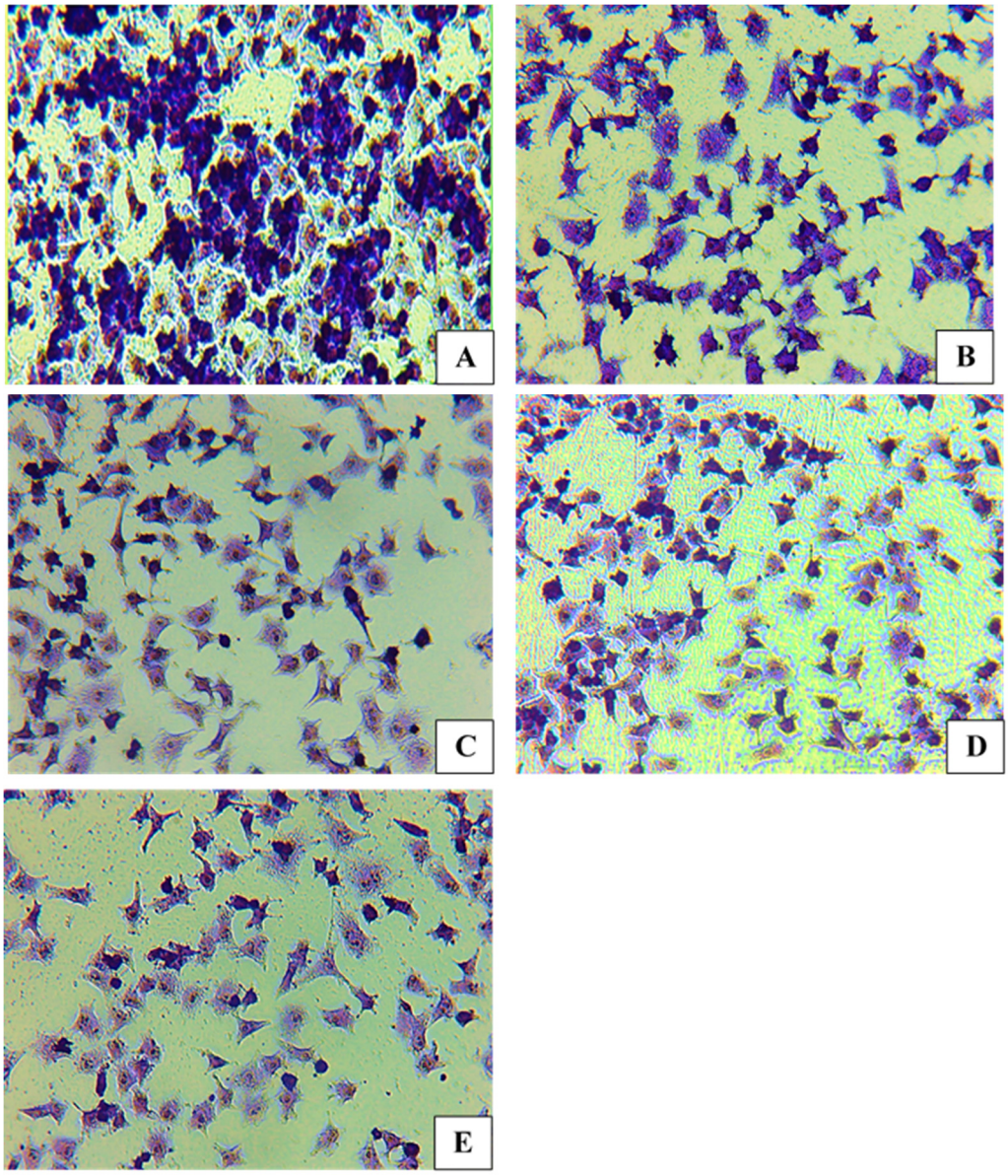

***Figure 3**. Histological sections of THP-1 cells stained with crystal violet after 24h exposure to 200 µg/mL of different P. quinquefolium extracts. (A) Control cells: formed dense clusters, indicating active proliferation. (B) Water extract: partially reduced cell numbers. (C) Hexane extract: further decreased cell density, (D) Ethanol extract: showed a stronger suppressive effect. (E) Chloroform extract: produced the lowest cell count, demonstrating the most pronounced inhibition of THP-1 cell survival.Scale bar = 100 µm. Magnification: (40*10x) 400x.*

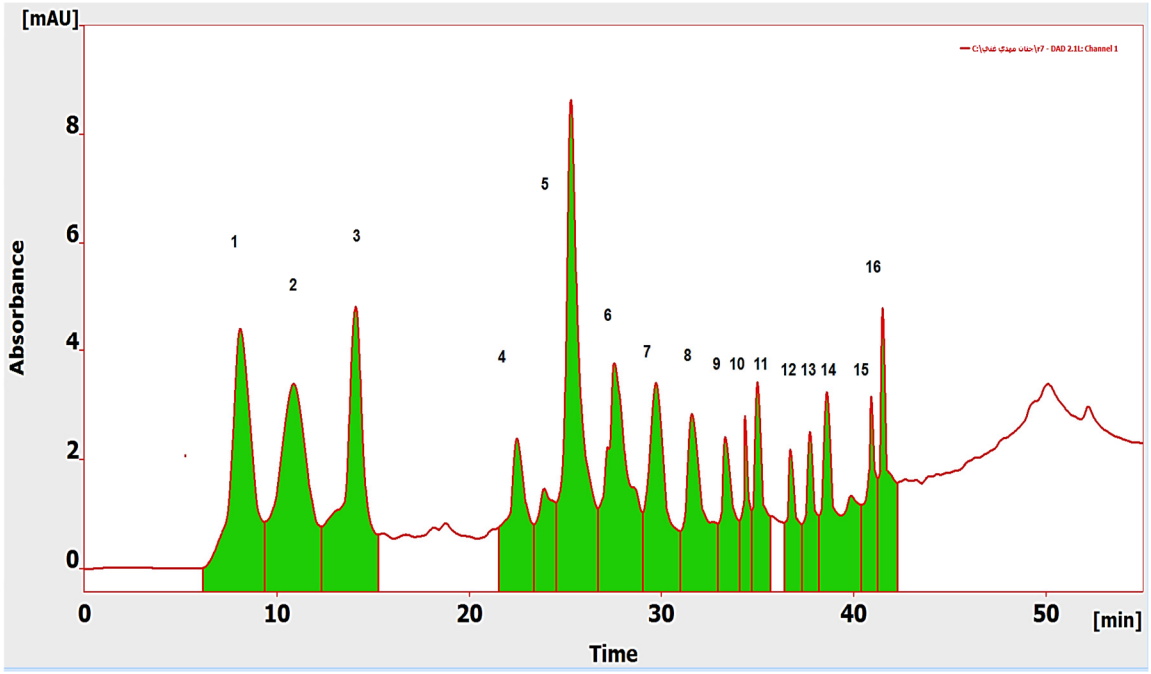

Figure 4: *HPLC Chromatogram of Chloroform Analysis and Peak Identification of Phytochemicals in Chloroform -ginseng extract.*