

Molecular Characterization and *In Vitro* Evaluation of Antioxidant and Cytotoxic Activities of *Echinacea purpurea* Aqueous Extracts from Different Sources

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Abstract

Objective: This study aimed to characterize the genetic diversity of *Echinacea purpurea* aqueous extracts obtained from three commercial sources and to evaluate the antioxidant and *in vitro* cytotoxic activities of the selected extract against human cancer cell lines.

Methods: Genetic variation among three *Echinacea purpurea* sources (A1: SWANSON, USA; A2: EMTENAN, Egypt; A3: local herbalist, Egypt) was assessed using Start Codon Targeted (SCoT) PCR molecular markers. The aqueous extract of Sample A1 was selected for biological testing based on genetic profiling. Antioxidant activity was evaluated using DPPH and ABTS radical scavenging assays. Cytotoxic activity was assessed against HCT-116 (colorectal carcinoma), HeLa (cervical adenocarcinoma), and MCF-7 (breast adenocarcinoma) cell lines using the MTT assay after 24 h treatment. The IC₅₀ was determined by log-linear regression, with R² and 95% confidence intervals calculated in Microsoft Excel.

Results: SCoT markers revealed only minor genetic variation among the three sources, supporting their overall molecular similarity. The aqueous extract demonstrated concentration-dependent radical scavenging activity in both antioxidant assays. Cytotoxicity testing revealed IC₅₀ values of $5,955.9 \pm 203.47$ µg/mL (R² = 0.9696; 95% CI: 5,391.0–6,520.8 µg/mL) for HCT-116 and $6,853.25 \pm 311.69$ µg/mL (R² = 0.9998; 95% CI: 5,988.2–7,718.3 µg/mL) for MCF-7. The HeLa cell line showed the lowest sensitivity, with 36.26% inhibition at 8,000 µg/mL and an IC₅₀ exceeding the tested range (>8,000 µg/mL; R² = 0.9798).

Conclusion: Aqueous *Echinacea purpurea* extracts from different commercial sources share high genetic similarity and exhibit measurable antioxidant and concentration-dependent cytotoxic activity. The modest cytotoxicity observed at high concentrations warrants further investigation using multiple independent experiments and mechanistic assays to establish biological relevance.

Keywords: Aqueous extract of *Echinacea*, molecular assessment, SCoT PCR, anti-tumor, HeLa cell line, antioxidant, ABTS, CUPRAC

Introduction

Although a comprehensive study integrating whole-genome sequencing, genetic variation mapping, and multi-omics analysis to directly associate the bioactivity of *Echinacea purpurea* aqueous extract with specific genetic variations in antioxidant defense, tumor suppression, and immune signaling pathways has not been documented in the current scientific literature as a cohesive investigation, numerous individual studies have examined these distinct aspects. *Echinacea purpurea* is a well-regarded medicinal plant esteemed for its varied pharmacological properties, including immunomodulatory, anti-inflammatory, antioxidant, and potential anticancer effects, primarily attributed to its abundant phytochemical constituents such as phenolic compounds and polysaccharides.^{1,2}

Aqueous extracts of *E. purpurea* exhibit notable biological activity. For instance, an aqueous extract (AE) of *E. purpurea* flowers has been shown to enhance wound healing by targeting the TGF-β/VEGF/NF-κB inflammatory pathway and exhibiting antioxidant activity.³ Further research highlighted the multifaceted protective effects of *E. purpurea* water extract against mercury(II) chloride toxicity in *Allium cepa* L., demonstrating its antioxidant and anti-tumor properties. Aqueous extracts have been chemically characterized to include diverse phenolic chemicals that contribute to their biological effects.⁴

The antioxidant defense systems regulated by *E. purpurea* extracts are thoroughly documented. Phenolic acids are recognized as the primary active compounds responsible for the antioxidant action of *E. purpurea*.⁵ Extracts exhibit

considerable antioxidant capacity through mechanisms such as DPPH radical scavenging and ferric-reducing antioxidant power. Antioxidant capacity is essential for alleviating oxidative stress, a state closely linked to the development of many illnesses.³ The Nrf2-Keap1 pathway is a vital regulator of cellular defense against oxidative and electrophilic stress, controlling the expression of antioxidant enzymes. Polysaccharides from *E. purpurea* have been shown to mitigate oxidative stress-induced hepatic damage by facilitating Parkin-dependent autophagy, which is associated with antioxidant response pathways.⁶ Although not explicitly mentioned in *E. purpurea* aqueous extracts, certain plant polysaccharides influence hepatocellular carcinoma by modulating the gut microbiota to inhibit the TLR4/NF-κB pathway, which is pertinent given the interplay between inflammatory and oxidative stress pathways. The Nrf2/Keap1 pathway is pivotal in mitigating arsenic-induced oxidative stress, and its activation by several polysaccharides has been identified as a fundamental biological mechanism for addressing oxidative stress-related illnesses.⁷

E. purpurea has demonstrated potential tumor-inhibition actions against many malignancies. A homogeneous polysaccharide from *E. purpurea* (EPPA) demonstrated anti-tumor activity by promoting M1 macrophage polarisation, a crucial mechanism in immune-mediated tumor suppression.⁸ A separate study illustrated the inhibitory effect of *E. purpurea* on hepatocellular carcinoma (HCC) in a murine model, with ongoing efforts to identify the active constituents and molecular basis for this activity using metabolomics and molecular docking.⁹ Network pharmacological analysis

and experimental validation have demonstrated the anti-lung cancer effects of *Echinacea* extracts, indicating greater anti-tumor potential. Particular extracts of *Echinacea purpurea* have demonstrated the ability to inhibit microbial strains and have anticancer properties. The Nrf2 pathway, largely recognized for its antioxidant defense, also exhibits a multifaceted role in cancer, sometimes functioning as a tumor suppressor and at other times facilitating tumor progression, contingent on the setting.¹⁰

Genetic characterization studies in *E. purpurea* have primarily focused on elucidating its metabolic pathways and responses to environmental stress. Transcriptomic and metabolomic methodologies have been employed to examine the mechanisms underlying biochar-induced effects, revealing alterations in plant development and nutritional status. Transcriptomics and metabolomics have elucidated the molecular defense mechanisms of *E. purpurea* against polycyclic aromatic hydrocarbon (PAH) pollution, identifying genes and metabolites implicated in detoxification and stress responses.¹¹ Expression Quantitative Trait Loci (eQTLs) mapping can statistically associate genetic variations with alterations in gene expression, therefore establishing a connection between genetic variation and functional consequences. An integrated genetic characterization would yield essential insights to optimize *E. purpurea* production and extract preparation, thereby enhancing medicinal effectiveness.¹²

This study aimed to evaluate the molecular efficacy of daily consumption of aqueous *Echinacea purpurea* extracts, with particular emphasis on their antioxidant capacity and tumor-inhibition potential. In parallel, genetic profiling was performed using Start Codon Targeted (SCoT) markers to assess genetic diversity among commercially available *Echinacea purpurea* samples obtained from local herbal markets and pharmaceutical companies, in order to determine potential differences in genetic composition that may influence bioactive consistency.

Materials and Methods

Plant Material and Extraction

Three distinct *Echinacea* samples were procured from various suppliers: SWANSON (North Dakota, USA; Sample A1), EMTENAN (Egypt; Sample A2), and a commercial herbalist in Egypt (Sample A3). To prepare the aqueous extracts, dried *Echinacea* leaves were infused in freshly boiled tap water at a concentration of 2 g/100 mL, which reflects a ratio commonly used in herbal tea preparation. The infusions were allowed to steep for 30 minutes at ambient temperature. Subsequently, the solutions were filtered through 0.22 µm Millex®-GP filters (Millipore, Bedford, MA), transferred into sterile containers, and stored at -20°C for subsequent analysis.¹³

Molecular Characterization

SCoT-PCR Amplification

DNA was extracted from the dried *Echinacea* leaf samples utilizing the DNeasy Plant Mini Kit (QIAGEN) according to the manufacturer's protocol. The isolated DNA served as a template for molecular characterization via Start Codon Targeted (SCoT) Polymerase Chain Reaction (PCR). Eleven

SCoT primers were employed to assess genetic polymorphism (Table 1). PCR amplification was conducted in a total reaction volume of 20 µL, comprising 10 µL of Master Mix (Sigma), 2 µL of primer (10 pmol), 2 µL of template DNA (10 ng), and 6 µL of nuclease-free water.¹⁴

Sample A1 (SWANSON, North Dakota, USA) was selected for biological evaluation based on its high genetic similarity to the other samples as determined by SCoT marker analysis, and its status as a commercially standardized pharmaceutical-grade supplement, ensuring consistent extract composition. The selection was based on two complementary criteria: (1) genetic similarity analysis using SCoT markers demonstrated that Sample A1 and Sample A2 exhibited the highest degree of genetic similarity (Dice's similarity coefficient of 92%); and (2) Sample A1 (SWANSON, North Dakota, USA) is a commercially standardized, internationally recognized pharmaceutical-grade *Echinacea* supplement, which provided a reliable and compositionally traceable reference material.

Thermocycling Conditions

Amplification was performed using a Perkin-Elmer/Gen-Amp® PCR System 9700 thermal cycler (PE Applied Biosystems). The cycling profile included an initial denaturation at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 45 s, annealing at 50°C for 50 s, and extension at 72°C for 1 min. A final extension step was carried out at 72°C for 7 min to ensure complete synthesis of all amplicons.¹⁵

Data Analysis and Phylogeny

For the SCoT analysis, only distinct and unequivocal bands were visually scored as either present (1) or absent (0) across all samples. The resulting dataset, comprising both polymorphic and monomorphic bands, was used to construct a binary statistical matrix. Dice's similarity coefficients were subsequently calculated between genotypes, and a phylogenetic tree (dendrogram) was generated using the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) algorithm. This

Table 1. List of Start Codon Targeted (SCoT) primers used for molecular characterization of *Echinacea* samples

Primer code	Sequence (5' to 3')
SCoT-1	ACGACATGGCGACCACGC
SCoT-2	ACCATGGCTACCACCGGC
SCoT-3	ACGACATGGCGACCCACA
SCoT-4	ACCATGGCTACCACCGCA
SCoT-5	CAATGGCTACCACTAGCG
SCoT-6	CAATGGCTACCACTACAG
SCoT-7	ACAATGGCTACCACTGAC
SCoT-8	ACAATGGCTACCACTGCC
SCoT-9	ACAATGGCTACCACCAGC
SCoT-10	ACAATGGCTACCACTACC
SCoT-11	ACAATGGCTACCACCAGC

dendrogram was based on the Euclidean similarity index and constructed using PAST software Version 1.91.¹⁶

In Vitro Cytotoxicity Assays

Cell Lines and Culture Conditions

Human colorectal carcinoma (HCT-116), breast adenocarcinoma (MCF-7), and cervical adenocarcinoma (HeLa) cell lines were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). Cells were maintained in RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50 µg/mL gentamicin. Cultures were incubated at 37°C in a humidified atmosphere containing 5% CO₂ and subcultured 2–3 times per week.^{17,18}

Cell Viability Analysis (MTT Assay)

Cytotoxicity was evaluated using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Cells were seeded at a density of 5×10^4 cells/well in 96-well plates and incubated for 24 h. Subsequently, cells were treated with 12 different concentrations of the *Echinacea* extracts in technical triplicate (three replicate wells per concentration) in a single independent experiment. After 24 h of exposure, the medium was replaced with 100 µL of fresh phenol-red-free RPMI-1640, and 10 µL of a 12 mM MTT stock solution (5 mg/mL in PBS) was added to each well. Following a 4-hour incubation at 37°C, the medium was removed, and 50 µL of DMSO was added to dissolve the formazan crystals. Optical density (OD) was measured at 590 nm using a microplate reader (SunRise, TECAN, Inc., USA).^{19,20} Cell viability was calculated as follows:

$$\% \text{ Viability} = (\text{OD}_{\text{treated}} / \text{OD}_{\text{control}}) \times 100$$

The 50% inhibitory concentration (IC₅₀) was determined using log-linear regression of percentage cell viability against the log₁₀ of concentration, applied to the active concentration range (500–8000 µg/mL; $n = 5$ data points per cell line). R² values and 95% confidence intervals were calculated in Microsoft Excel.^{19,20}

In Vitro Antioxidant Activity

DPPH Radical Scavenging Assay

The free radical scavenging capacity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Various concentrations of the extract (0–1000 µg/mL) were mixed with 2.4 mL of 0.1 mM DPPH in methanol. After 30 min of incubation in the dark at room temperature, absorbance was measured at 517 nm. Ascorbic acid served as the positive control.²¹ Scavenging activity was calculated using the equation:

$$\% \text{ Scavenging} = [(A_0 - A_1) / A_0] \times 100$$

where A₀ is the absorbance of the control, and A₁ is the absorbance of the sample.²²

ABTS Radical Scavenging Assay

The ABTS•+ scavenging activity was determined according to the method of Re et al. with minor modifications. The ABTS radical cation was generated by reacting 7 mM ABTS

with 2.45 mM potassium persulfate for 12–16 h in the dark. The solution was diluted to an absorbance of 0.70 ± 0.02 at 734 nm. The reaction mixture, containing 3 mL of ABTS•+ and 0.07 mL of extract, was incubated for 1 h before measuring absorbance at 734 nm.²³

$$\text{ABTS radical scavenging ability (\%)} = (A_0 - A_1 + A_2) \times 100 / A_0$$

Hydrogen Peroxide (H₂O₂) Scavenging Assay

The H₂O₂ scavenging ability was evaluated using the method of Ruch et al. Extracts (0–1000 µg/mL) were mixed with phosphate buffer and 600 µL of a 40 mM H₂O₂ solution. After 10 min, absorbance was recorded at 230 nm against a phosphate buffer blank. Ascorbic acid was used as the standard. The ability to scavenge hydrogen peroxide was determined using the equation.²⁴

$$\% \text{ scavenged (H}_2\text{O}_2) = (A_0 - A_1) / A_0 \times 100 \quad (2)$$

where A₁ is the sample's absorbance, and A₀ is that of the control.

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was used to quantify the ferric-reducing capacity. The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ, and 20 mM FeCl₃•6H₂O in a 10:1:1 ratio. Extracts (0–1000 µg/mL) were incubated with 3 mL of FRAP reagent at 37°C for 30 min. The increase in absorbance at 593 nm was measured and calibrated against FeSO₄ standards.²⁵

Cupric Ion Reducing Antioxidant Capacity (CUPRAC)

The CUPRAC assay was performed by mixing 1 mL each of CuCl₂ (10 mM), neocuproine (7.5 mM), and ammonium acetate buffer (1 M, pH 7.0) with 0.5 mL of the sample. Following a 30-minute incubation, absorbance was measured at 450 nm. Results were expressed as milligrams of Trolox equivalents per gram (mg TE/g).²⁶

Metal Chelating Activity

The ferrous ion chelating capacity was assessed by mixing 0.1 mL of the extract with 2 mM FeCl₂. The reaction was initiated by adding 0.2 mL of 5 mM ferrozine. After 10 min of incubation, absorbance was measured at 562 nm. EDTA served as the positive control.²⁷ The formula was used to calculate the percentage of inhibition of Ferrozine-Fe²⁺ complex formation.

$$I = (\text{Abs control} - \text{Abs sample}) / \text{Abs control} \times 100$$

EDTA was used as a positive control.²⁸

Statistical Analysis

Data processing and calculations for scavenging activities were conducted in Microsoft Excel. IC₅₀, P-value, t-Test, and R² values were calculated in Excel. R² values reported for antioxidant dose-response curves were derived from linear regression of concentration versus percentage scavenging activity. Low R² values in certain assays reflect non-linear behavior of the extract at tested concentrations, indicating that a linear

model was not optimal for those datasets; this is noted as a limitation in the respective Results subsections. Results are presented as mean $\pm$ standard deviation (SD).²⁹

Results

SCoT Polymorphism Analysis

Start codon targeted (SCoT) polymorphism, a gene-targeted marker, focuses on the conserved region adjacent to the translation initiation start codon (ATG) in plant genes.³⁰ In this investigation, 11 SCoT primers were used, yielding 136 bands. Of these, 29% ($n = 29$) exhibited polymorphism, while 107 bands were monomorphic. The mean number of bands per primer was 12.4, with 9.7 monomorphic and 2.7 polymorphic bands. Primer 11 SCoT generated the most polymorphic bands, whereas SCoT-02 produced the largest overall number of bands. Conversely, SCoT-10 displayed the highest count of monomorphic bands. The polymorphism percentage ranged from 14% (SCoT-10) to 33% (SCoT-01), with an overall average of 22% (Table 2).

Three *Echinacea* samples were molecular fingerprinted: A1, sourced from the SWANSON business in North Dakota, USA; A2, obtained from the EMTENAN company in Egypt; and A3, acquired from a commercial herbalist shop in Egypt. The similarity matrices for these samples are presented in Table 3. A UPGMA dendrogram (Figure 1) indicated a closer genetic relationship between samples A1 and A2 than between A3 and the other samples. Specifically, the similarity between A1 and A2 was 92%, and between A1 and A3, 91.7%. The similarity between A2 and A3 was 91.1%. All 11 SCoT primers consistently produced highly scorable and reproducible bands (Figure 2). Notably, no substantial variation was observed among *Echinacea* sources.

Anti-tumor Activity

Numerous natural compounds derived from plants and herbs have demonstrated anti-cancer properties, including

cytotoxicity, inhibition of proliferation, induction of apoptosis, suppression of metastasis, inhibition of angiogenesis, and enhancement of chemotherapeutic efficacy.³¹ The anti-cancer efficacy of an aqueous *Echinacea* extract was investigated against HCT-116, HeLa, and MCF-7 cell lines. The *Echinacea* aqueous extract (SWANSON (North Dakota, USA; Sample A1), which was chosen) was tested at a concentration of 8000 $\mu\text{g/ml}$, and the results are summarized in Table 4. Previous studies have also highlighted the significant antiproliferative properties of *Echinacea* aqueous extracts.^{31,32}

The antitumor activity of the aqueous extract of *Echinacea* was evaluated against HCT-116, HeLa, and MCF-7 human cancer cell lines using the MTT assay following 24 h of treatment. The extract exhibited concentration-dependent cytotoxic effects, with varying degrees of sensitivity among the tested cell lines. At the highest tested concentration (8000 $\mu\text{g/ml}$), the extract exhibited inhibition rates of 43.28%, 63.74%, and 46.21% against HCT-116, HeLa, and MCF-7 cells, respectively, with corresponding cell viabilities of 56.72%, 36.26%, and 53.79% (Table 4). These findings indicate that HeLa cells were the most susceptible to the extract, whereas HCT-116 and MCF-7 cells exhibited moderate sensitivity. The differences in cytotoxic responses were further reflected in the IC_{50} values as shown in Table 5. Table 6 shows the IC_{50} values, goodness-of-fit (R^2), 95% confidence intervals, and log-linear regression equations for the cytotoxic activity of aqueous *Echinacea*

Table 3. The similarity matrix of the three samples of *Echinacea*: A1 from the SWANSON company in North Dakota, USA, A2 from the EMTENAN company in Egypt, and A3 from a commercial herbalist store in Egypt

	A1	A2	A3
A1	100		
A2	92.0	100	
A3	91.7	91.1	100

Table 2. The list of primers, Total Number of Bands (TB), Monomorphic Bands (MB), Polymorphic Bands (PB), and Percentage of Polymorphism (%P) as revealed by SCoT analysis 3 for *Echinacea* three samples

Primer	Total Bands (TB)	Monomorphic Bands (MB)	Polymorphic Bands (PB)	% Polymorphism (%P)
SCoT-01	12	8	4	33
SCoT-02	9	6	3	33
SCoT-03	13	11	2	15
SCoT-04	16	11	5	31
SCoT-05	13	11	2	15
SCoT-06	12	10	2	17
SCoT-07	10	8	2	20
SCoT-08	12	10	2	17
SCoT-09	13	11	2	15
SCoT-10	14	12	2	14
SCoT-11	12	9	3	25
Total	136	107	29	—
Average	12.4	9.7	2.7	22

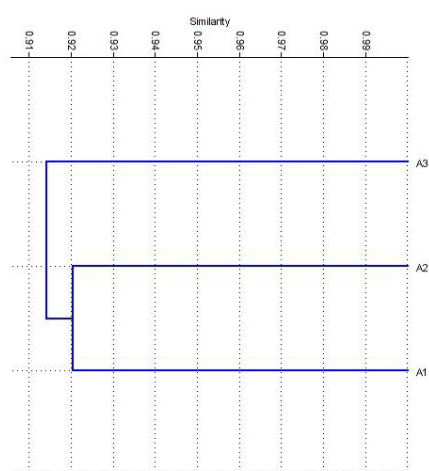


Fig. 1 The dendrogram of the similarity matrix of the three samples of *Echinacea*: A1 from the SWANSON company in North Dakota, USA, A2 from the EMTENAN company in Egypt, and A3 from a commercial herbalist store in Egypt.

purpurea extract against HCT-116 (human colorectal carcinoma), HeLa (human cervical adenocarcinoma), and MCF-7 (human breast adenocarcinoma) cell lines, as determined by the MTT assay after 24 h treatment. IC_{50} values were derived from log-linear regression of percentage cell viability against $\log_{10}(\text{concentration})$ across the active concentration range (500–8,000 $\mu\text{g/mL}$; $n = 5$ data points per cell line). The 95% confidence interval was calculated using a t -distribution with $df = 3$ (t -critical = 2.776). For the HeLa cell line, 50% inhibition was not achieved within the tested concentration range; the IC_{50} is therefore reported as $>8,000$ $\mu\text{g/mL}$ and no confidence interval is applicable. SD: standard deviation; R^2 : coefficient of determination; CI: confidence interval. Overall, the aqueous extract displayed measurable antiproliferative activity, particularly against HeLa cells, although its potency was considerably lower than that of the standard chemotherapeutic agent (Figures 3 and 4). The absence of a determinable IC_{50} for the HeLa cell line within the tested concentration range does not indicate a lack of cytotoxic effect; rather, it reflects that the extract concentration required to inhibit 50% of cell viability exceeds

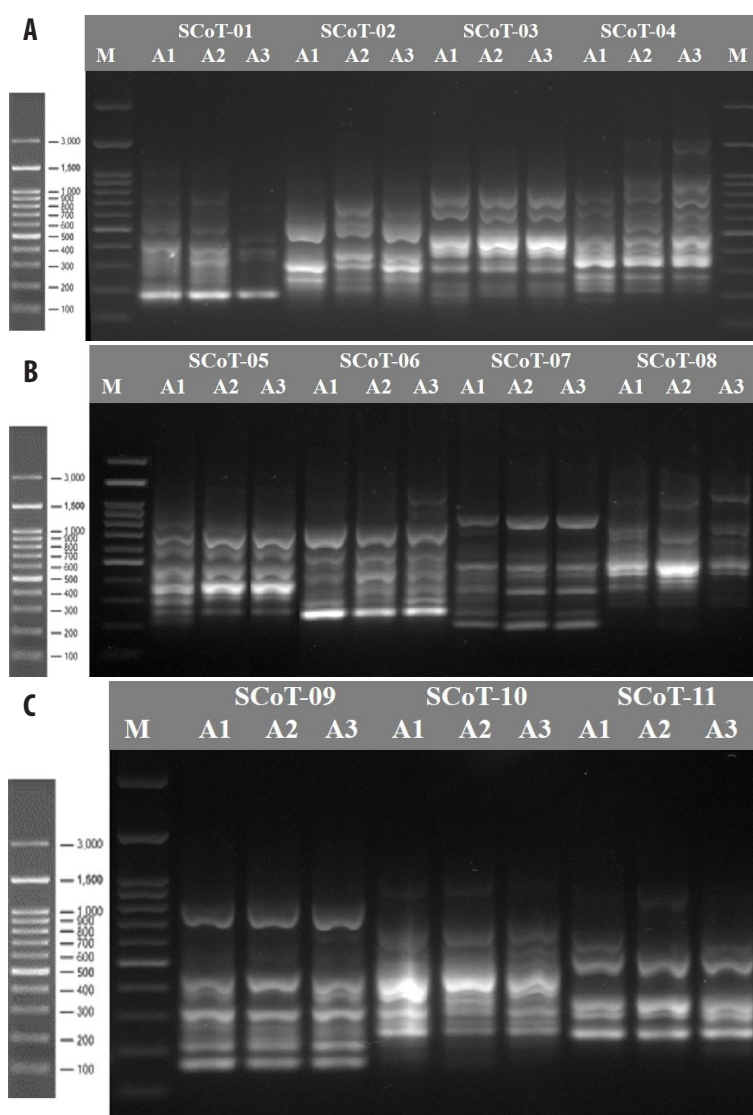


Fig. 2 Amplification profile generated through SCoT markers in the three sources of *Echinacea*. A): The three sources of *Echinacea* with SCoT-01, SCoT-02, SCoT-03, and SCoT-04, B): The three sources of *Echinacea* with SCoT-05, SCoT-06, SCoT-07 and SCoT-08, C): The three sources of *Echinacea* with SCoT-09, SCoT-10 and SCoT-11. M represents a 3000 bp molecular weight ladder.

Table 4. Inhibition rates at 8000 µg/ml of the *Echinacea* aqueous extract in three cancer cell lines. (The treatment time used in cytotoxicity studies is 24 h)

The <i>Echinacea</i> aqueous extract	HCT-116	HeLa	MCF-7
Inhibitory	43.28%	63.74%	46.21%
Viability	56.72%	36.26%	53.79%
S.D. (±)	3.16	2.12	3.45

Table 5. IC₅₀ value (µg/ml) of the screened *Echinacea* aqueous extract (8000 µg/ml) and cisplatin (the control) in three cancer cell lines, using MTT assay. (The treatment time used in cytotoxicity studies is 24 h)

Compounds	HCT-116	HeLa	MCF-7
The <i>Echinacea</i> aqueous extract (8000 µg/ml)	5955.9 ± 203.47	>8000	6853.25 ± 311.69
Cisplatin (1000 µg/ml)	2.53 ± 0.21	7.59 ± 0.62	5.71 ± 0.34

8,000 µg/mL under the experimental conditions employed. The observed 36.26% inhibition at the maximum tested concentration of 8,000 µg/mL confirms a measurable, dose-dependent reduction in cell viability, consistent with the regression model ($R^2 = 0.9798$), yet insufficient to reach the 50% inhibition threshold. These findings suggest that HeLa cells exhibit comparatively lower sensitivity to the aqueous *Echinacea purpurea* extract relative to HCT-116 and MCF-7 cell lines. **Supplementary Tables 1 and 2** showed the *t*-test of the three cell lines' results, including the mean, Variance, Observations, Correlation, Hypothesized Mean, Difference, df, *t*-stat, *P*-value ($T \leq t$) one-tail, *t* Critical one-tail, *P*-value ($T \leq t$) two-tail, and *t* Critical two-tail results, and the raw data of the viability, inhibition ratio, and S.D. (±) of HCT-116, HeLa, and MCF-7 cell lines.

Antioxidant activity

Natural plant compounds are recognized for their capacity to scavenge free radicals, which are implicated in cellular apoptosis, histological damage, and the pathogenesis of chronic diseases.³³ Although numerous studies have evaluated the antioxidant properties of *Echinacea* extracts using organic

Table 6. IC₅₀ values, goodness-of-fit (R^2), 95% confidence intervals, and log-linear regression equations for the cytotoxic activity of aqueous *Echinacea purpurea* extract (Sample A1) against HCT-116 (human colorectal carcinoma), HeLa (human cervical adenocarcinoma), and MCF-7 (human breast adenocarcinoma) cell lines, as determined by the MTT assay after 24 h treatment

Cell line	IC ₅₀ (µg/mL)	SD (±)	R^2	95% CI (µg/mL)	Regression equation
HCT-116	5,955.9	±203.47	0.9696	5,391.0 – 6,520.8	Viability = $-43.22 \times \log_{10}(\text{Conc}) + 211.29$
HeLa	>8,000	—	0.9798	Not applicable	Viability = $-29.39 \times \log_{10}(\text{Conc}) + 176.84$
MCF-7	6,853.25	±311.69	0.9998	5,988.2 – 7,718.3	Viability = $-43.81 \times \log_{10}(\text{Conc}) + 217.10$

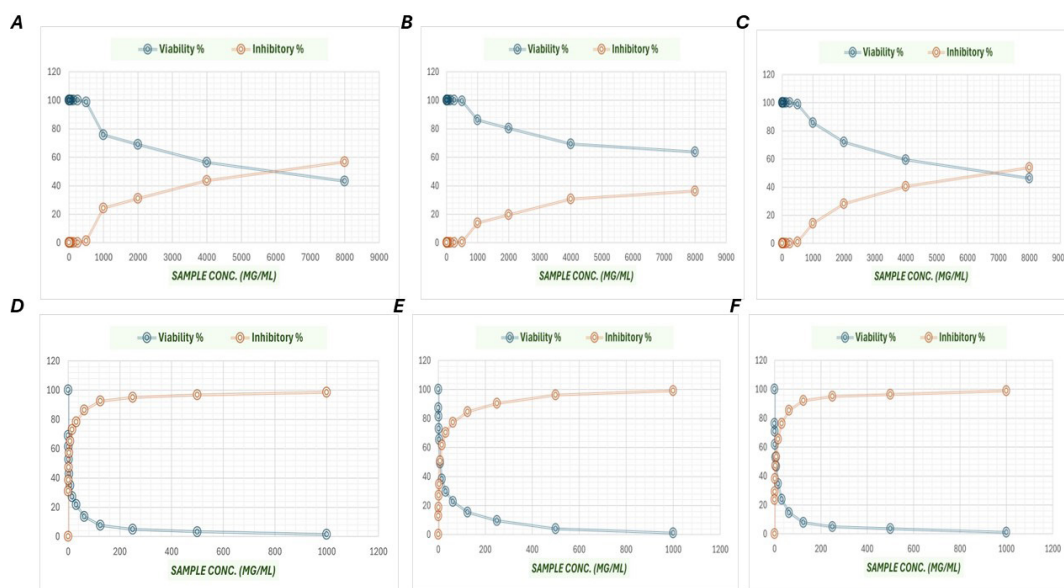


Fig 3. Cytotoxicity of the aqueous extract of *Echinacea* and cisplatin (8000 µg/ml) on the three cell lines, including the percentage of viability and inhibition. The effect of the aqueous extract of *Echinacea* on cell growth and viability was shown against (A): the HCT-116 cell line, (B) the HeLa cell line, and (C): the MCF-7 cell line. Also, the effect of cisplatin on cell growth and viability was shown against (D) the HCT-116 cell line, (E) the HeLa cell line, and (F) the MCF-7 cell line.

solvents or ethanol, comparatively fewer studies have specifically characterized the antioxidant activity of aqueous (tea-style infusion) preparations across multiple complementary assays simultaneously. The present study addresses this gap by applying six antioxidant assays to an aqueous *Echinacea* infusion prepared under conditions mimicking typical herbal tea consumption. Evaluating the antioxidant efficacy

of *Echinacea* extract is crucial for its potential applications in the food, pharmaceutical, and cosmetics industries. A diverse array of methodologies exists for assessing compound antioxidant activity, reflecting the various mechanisms underlying it. For this research, the following assays were selected: DPPH, ABTS radical scavenging, H_2O_2 scavenging, FRAP scavenging, CUPRAC assay, and metal chelating activity.³⁴ Ascorbic acid served as a positive control (Figure 5).

The antioxidant potential of the aqueous extract of *Echinacea* (1000 $\mu\text{g/ml}$) was comprehensively evaluated using six complementary in vitro assays, including DPPH, ABTS, hydrogen peroxide (H_2O_2) scavenging, ferric reducing antioxidant power (FRAP), cupric ion reducing antioxidant capacity (CUPRAC), and metal-chelating activity. The extract exhibited strong antioxidant activity across all tested assays, with IC_{50} values of 6.89 ± 0.43 , 3.72 ± 0.23 , 11.50 ± 1.23 , 14.89 ± 2.03 , 24.33 ± 1.68 , and 29.33 ± 2.04 $\mu\text{g/ml}$ for DPPH, ABTS, H_2O_2 , FRAP, CUPRAC, and metal-chelating assays, respectively (Figure 5 and Table 7). In comparison with the corresponding reference antioxidants, the extract demonstrated superior radical scavenging activity in the DPPH, ABTS, H_2O_2 , and FRAP assays, while exhibiting stronger cupric ion reducing capacity than EDTA but a slightly lower metal-chelating activity. The total antioxidant capacity (TAC) of the extract reached 61.38 ± 3.14 mg GAE/g, representing approximately 83% of the antioxidant capacity of ascorbic acid (74.19 ± 3.97 mg GAE/g) (Table 8). Consistent with these findings, the extract achieved high antioxidant inhibition percentages ranging from 89.56% to 95.42% across the different assays,

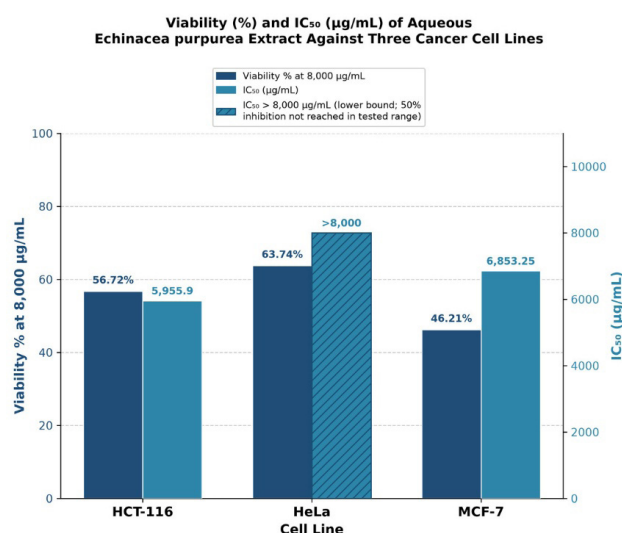


Fig. 4 The viability and IC_{50} of the aqueous extract of *Echinacea* (8000 $\mu\text{g/ml}$) against the HCT-116, HeLa, and MCF-7 cell lines.

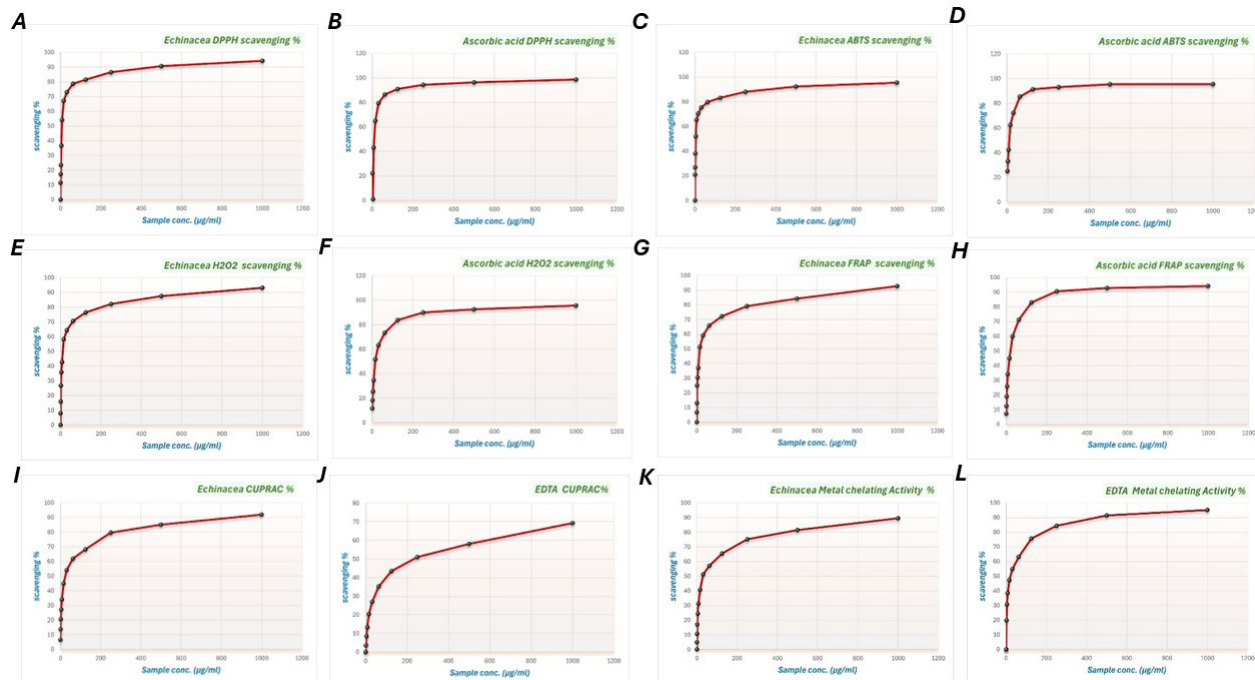


Fig. 5 The antioxidant activity of the aqueous extract of *Echinacea* (1000 $\mu\text{g/ml}$) in comparison with the control. A): using DPPH scavenging with echinacea, B): using DPPH scavenging with Ascorbic acid control, C): using ABTS radical scavenging with echinacea, D): using ABTS radical scavenging with Ascorbic acid control, E): using H_2O_2 Scavenging Assay with echinacea, F): using H_2O_2 Scavenging Assay with Ascorbic acid control, G): using FRAP Scavenging Assay with echinacea, H): using FRAP Scavenging Assay with Ascorbic acid control, I): using CUPRAC Assay with echinacea, J): CUPRAC Assay with EDTA, K): using Metal chelating Activity Assay with echinacea, L): using Metal chelating Activity Assay with EDTA.

Table 7. The IC₅₀ value (1000 µg/ml) resulted from the antioxidant activity of the screened *Echinacea* aqueous extract

Compounds	DPPH scavenging %	ABTS	H ₂ O ₂	FRAP	CUPRAC	Metal chelating activity
The <i>Echinacea</i> aqueous extract	6.89 ± 0.43 µg/ml	3.72 ± 0.23 µg/ml	11.50 ± 1.23 µg/ml	14.89 ± 2.03 µg/ml	24.33 ± 1.68 µg/ml	29.33 ± 2.04 µg/ml
EDTA positive control	–	–	–	–	232.28 ± 4.73	21.16 ± 2.08 µg/ml
Ascorbic acid standard	10.21 ± 0.77	10.66 ± 0.89	14.77 ± 0.69	20.89 ± 1.25	–	–

Table 8. The TAC (Total Antioxidant Capacity) and (±) SD of the aqueous extract of *Echinacea* (1000 µg/ml) and Ascorbic acid

Sample code	TAC (mg GAE/g)	(±) SD
<i>Echinacea</i>	61.38	3.14
Ascorbic acid (Positive control)	74.19	3.97

Table 9. The percentage of antioxidant activity for the *Echinacea* sample (1000 µg/ml) and the control was determined using DPPH, ABTS, H₂O₂, FRAP, CUPRAC, and metal-chelating Activity Assays

Sample code	DPPH	ABTS	H ₂ O ₂	FRAP	CUPRAC	Metal chelating
<i>Echinacea</i>	94.21	95.42	93.06	92.75	91.87	89.56
Control	98.65	95.46	95.73	94.19	69.32	95.13

confirming its broad-spectrum antioxidant potential (Table 9).

Discussion

Herbal infusions have held a central position in traditional medicine for over two millennia, with historical records documenting their systematic use in ancient China as broad-spectrum therapeutic agents.³⁵ Although initially confined to Asian societies, herbal teas were introduced to Europe during the seventeenth century through transcontinental exploration, where they rapidly gained acceptance.³⁶ In contemporary practice, herbal teas represent a globally consumed, year-round functional beverage category. Importantly, unlike infusions derived from *Camellia sinensis*, herbal teas are prepared from diverse medicinal plants and are naturally caffeine-free, thereby permitting frequent daily consumption without stimulant-associated adverse effects.³⁷

Among medicinal botanicals, the genus *Echinacea* (family Asteraceae) has attracted considerable scientific and commercial interest. *Echinacea* species are perennial plants taxonomically related to sunflowers, daisies, and ragweed, and their preparations are predominantly marketed as dietary supplements for upper respiratory infections and immune modulation.² Despite widespread consumption, rigorous evaluation of aqueous *Echinacea* infusions as standardized anti-tumor and antioxidant beverages remains insufficient. The present study

addresses this gap by comprehensively investigating the anti-tumor and antioxidant efficacy of *Echinacea* aqueous extract while concurrently assessing genetic characterization among different sources using Start Codon Targeted (SCoT) polymorphism analysis.

Genetic diversity assessment is critical to ensuring phytochemical consistency and reproducibility of therapeutic effects. The SCoT marker system, which targets conserved regions flanking the ATG start codon, has emerged as a robust, gene-targeted molecular fingerprinting tool in plant genetics and breeding due to its simplicity, cost-effectiveness, high polymorphism, reproducibility, and independence from prior sequence information.³⁸ In the present analysis, only minor genetic characterization was detected among the three *Echinacea* sources, suggesting a high degree of genetic similarity and supporting their potential to deliver comparable bioactive profiles. These findings are consistent with nuclear DNA content analyses reported by Jedrzejczyk,³⁹ which showed that most *Echinacea* species have intermediate genome sizes, with notable variation between species, such as *Echinacea pallida* (2C = 28.59 pg) and *Echinacea simulata* (2C = 14.31 pg). Collectively, our genetic data strengthen the reliability of the investigated sources and substantiate their suitability for comparative phytopharmacological evaluation.

The present findings demonstrate that the aqueous extract of *Echinacea* (source: SWANSON (North Dakota, USA) possesses selective antiproliferative activity against human cancer cell lines, with the highest inhibitory effect observed in HeLa cells. The variation in cytotoxic responses among the tested cell lines is likely attributable to differences in their genetic background, metabolic characteristics, membrane permeability, and susceptibility to apoptosis. Although the extract was less potent than cisplatin, this outcome is expected because aqueous plant extracts contain complex mixtures of active and inactive phytochemicals, resulting in lower concentrations of individual bioactive constituents than purified chemotherapeutic agents. Nevertheless, the observed cytotoxic activity suggests that the extract contains compounds capable of interfering with tumor cell proliferation. Previous studies have attributed the anticancer properties of *Echinacea* species to phenolic acids, flavonoids, caffeic acid derivatives, alkylamides, and polysaccharides, which have been reported to induce apoptosis, arrest the cell cycle, suppress oxidative stress, and modulate inflammatory signaling pathways associated with tumor progression. Therefore, the current results support the potential of *Echinacea* as a promising natural source of anticancer agents, although additional phytochemical isolation and mechanistic investigations are required to identify the constituents responsible for the observed biological activity.⁴⁰ Consistent with previous reports indicating dose-dependent reductions in cancer cell viability following *Echinacea* treatment.^{41,42}

Some studies note that *Echinacea* has a long-standing history as a therapeutic agent and is widely used in the treatment and prevention of upper respiratory tract infections. The extract of *E. purpurea* exhibited anti-tumor activity against the MCF7 breast cancer cell line. A 25 µg/mL *Echinacea* extract completely inhibited HeLa cells.⁴³ Our results align with other reports; however, higher doses were necessary to achieve similar biological action. The disparity is likely attributed to the use of an aqueous extract in the current study, whereas the prior research utilised a refined “*Echinacea purpurea*” extract, leading to variations in the concentration and bioavailability of bioactive phytochemicals.

The aqueous extract of *Echinacea* (1000 µg/ml) demonstrated potent antioxidant activity through multiple mechanisms, including free radical scavenging, reducing power, hydrogen peroxide neutralization, and transition metal ion chelation. The consistently low IC₅₀ values observed in the DPPH and ABTS assays indicate an efficient hydrogen- and electron-donating capacity, while the favorable performance in the FRAP and CUPRAC assays reflects a strong reducing potential. Furthermore, the substantial hydrogen peroxide scavenging and metal-chelating activities suggest that the extract may reduce oxidative damage by limiting the generation of highly reactive hydroxyl radicals through Fenton-type reactions. The high total antioxidant capacity and inhibition percentages across all assays further support the presence of abundant antioxidant phytochemicals within the extract. These activities are likely associated with the rich content of phenolic acids, flavonoids, caffeic acid derivatives, chicoric acid, chlorogenic acid, and other polyphenolic constituents previously identified in *Echinacea* species. Because oxidative

stress plays a central role in carcinogenesis through DNA damage, chronic inflammation, and dysregulation of cellular signaling pathways, the strong antioxidant properties observed in the present study may contribute to the antitumor effects demonstrated against the tested cancer cell lines. These findings are consistent with the work of Azar Sadeghi et al.,⁴³ who reported high phenolic content and strong radical-scavenging capacity in *Echinacea purpurea* tissues. Low R² values observed in certain dose-response curves suggest that the relationship between extract concentration and scavenging activity was not strictly linear across all tested concentrations. This non-linearity is acknowledged as a limitation and should be considered when interpreting the quantitative results.

The use of different concentration ranges for the antiproliferative and antioxidant assays reflects the distinct sensitivities and underlying mechanisms of these biological responses. While the cytotoxic activity was evaluated at concentrations up to 8000 µg/ml to achieve measurable growth inhibition, the antioxidant assays demonstrated substantial radical-scavenging activity at considerably lower concentrations (up to 1000 µg/ml), indicating that the antioxidant constituents of the aqueous extract remain highly effective even at reduced doses. Consequently, the antioxidant and antiproliferative activities of the *Echinacea* aqueous extract appear to be complementary biological properties that together support its potential as a natural source of bioactive compounds for future therapeutic development.

Conflicts of Interest

None. ■

References

- Latif, R. and T. Nawaz, Medicinal plants and human health: a comprehensive review of bioactive compounds, therapeutic effects, and applications. *Phytochemistry Reviews*, 2025. 25, p. 2299–2342.
- Ahmadi, F., et al., *Echinacea*: Bioactive Compounds and Agronomy. *Plants*, 2024. 13(9): p. 1235.
- Ezzat, M.I., et al., Targeting TGF-β/VEGF/NF-κB inflammatory pathway using the Polyphenols of *Echinacea purpurea* (L.) Moench to enhance wound healing in a rat model. *Inflammopharmacology*, 2025. 33(4): p. 2151–2164.
- Yalçın, E., et al., Multi-protective role of *Echinacea purpurea* L. water extract in *Allium cepa* L. against mercury(II) chloride. *Environ Sci Pollut Res Int*, 2021. 28(44): p. 62868–62876.
- Mei, B., et al., Changes of Phenolic Acids and Antioxidant Activities in Diploid and Tetraploid *Echinacea purpurea* at Different Growth Stages. *Revista Brasileira de Farmacognosia*, 2020. 30(4): p. 510–518.
- Adinolfi, S., et al., The KEAP1-NRF2 pathway: Targets for therapy and role in cancer. *Redox Biology*, 2023. 63: p. 102726.
- Chen, N., et al., Insights into the molecular mechanisms, structure-activity relationships and application prospects of polysaccharides by regulating Nrf2-mediated antioxidant response. *Carbohydrate Polymers*, 2024. 333: p. 122003.
- Ren, W., et al., *Echinacea purpurea*-derived homogeneous polysaccharide exerts anti-tumor efficacy via facilitating M1 macrophage polarization. *The Innovation*, 2023. 4(2): p. 100391.
- Xu, W., et al., Material basis research for *Echinacea purpurea* (L.) Moench against hepatocellular carcinoma in a mouse model through integration of metabolomics and molecular docking. *Phytomedicine*, 2022. 98: p. 153948.
- Ma, S.M., et al., Network Pharmacological Analysis and Experimental Validation of Anti-Lung Cancer Effect of *Echinacea Purpurea*. *Letters in Drug Design & Discovery*, 2025. 22.
- Tan, L., et al., Integrated Transcriptomic and Metabolomic Analysis Reveals Biochar-Induced Enhancement of Growth and Secondary Metabolism in the Medicinal Plant *Echinacea purpurea*. *Int J Mol Sci*, 2025. 26(23).
- Nica, A.C. and E.T. Dermizakis, Expression quantitative trait loci: present and future. *Philos Trans R Soc Lond B Biol Sci*, 2013. 368(1620): p. 20120362.
- Ziani, I., et al., Sustainable management of rosemary wastewater and essential oil in agri-environmental bioprocessing. *Food Bioscience*, 2024. 62: p. 105263.
- Chñapek, M., et al., Genetic diversity of maize resources revealed by different molecular markers. *Genetic Resources and Crop Evolution*, 2024. 71(8): p. 4685–4703.
- Lorenz, T.C., Polymerase chain reaction: basic protocol plus troubleshooting and optimization strategies. *J Vis Exp*, 2012(63): p. e3998.
- Habiba, R., et al., Assessment of Genetic Diversity Using SCOT Markers and Some Morphological Traits in Ten Lines of Barley (*Hordeum vulgare* L.). *Assiut Journal of Agricultural Sciences*, 2022. 52: p. 53–65.
- Pogorzelska, A., J. Ślawiński, and A. Kawiak, New N-Benzenesulfonylguanidine Derivatives and Their Selective Growth Inhibition of Human Breast Cancer Cell Line MCF-7 and Colon Carcinoma HCT-116. *Vol. 14*. 2022. 49.
- El Sadda, R.R., et al., Synthesis and biological evaluation of titanium dioxide/thiopolyurethane composite: anticancer and antibacterial effects. *BMC Chem*, 2024. 18(1): p. 35.
- Maray, S.O., et al., In Vitro Assessment of Antiviral, Antimicrobial, Antioxidant and Anticancer Activities of Ulvan Extracted from the Green Seaweed *Ulva lactuca*. *Thalassas: An International Journal of Marine Sciences*, 2023. 39(2): p. 779–790.
- Hossain, M.M., et al., Discovery of a novel and highly selective JAK3 inhibitor as a potent hair growth promoter. *Journal of Translational Medicine*, 2024. 22(1): p. 370.
- Vlase, A.-M., et al., *Epilobium* Species: From Optimization of the Extraction Process to Evaluation of Biological Properties. *Antioxidants*, 2023. 12(1): p. 91.
- Gulcin, İ. and S.H. Alwasel, DPPH Radical Scavenging Assay. *Processes*, 2023. 11(8): p. 2248.

23. R. Senthil kumar, A. Rajesh, L. Mullai Nathan, P.S. Tresina, V.R. Mohan Phytochemical, Antioxidant Property and Free Radical Scavenging Activity of *Henckelia humboldtiana* (Gardner) A. Weber & B.L. Burt (Gesneriaceae). *Journal of Pharmaceutical Negative Results*, 2023: p. 2072–2078.
24. Pleh, A., L. Mahmutović, and A. Hromić-Jahjefendić, Evaluation of phytochemical antioxidant levels by hydrogen peroxide scavenging assay. *Bioengineering Studies*, 2021. 2(1): p. 1–10.
25. Hussein Rassem, H., et al., Comprehensive analysis of antioxidant and antibacterial activities of water and methanol extracts of Hibiscus flower. *Journal of King Saud University - Science*, 2024. 36(11): p. 103506.
26. Yan, K., et al., The Influence of Different Extraction Techniques on the Chemical Profile and Biological Properties of *Oroxylum indicum*: Multifunctional Aspects for Potential Pharmaceutical Applications. *Evid Based Complement Alternat Med*, 2022. 2022: p. 8975320.
27. Khadhri, A., et al., Identification of Secondary Metabolites in Tunisian *Tilia platyphyllos* Scop. Using MALDI-TOF and GC-MS. *Journal of Renewable Materials*, 2024. 12(4): p. 827–842.
28. Tan, Y., et al., Impacts of Fermentation on the Phenolic Composition, Antioxidant Potential, and Volatile Compounds Profile of Commercially Roasted Coffee Beans. *Fermentation*, 2023. 9: p. 918.
29. Sundarapandiyan, V., et al., Chemical Characterization and Free Radical Scavenging Activity of *Dendrobium Thongchai* Extracts: A Promising Source for Neuroprotective and Antimicrobial Agents. *Asian Journal of Pharmaceutical and Clinical Research*, 2026: p. 175–181.
30. Sharma, V. and M. Thakur, Applicability of SCoT markers for detection of variations in *Fusarium* yellows resistant lines of ginger (*Zingiber officinale* Rosc.) induced through gamma irradiations. *South African Journal of Botany*, 2021. 140: p. 454–460.
31. Adico, M.D., et al., *In vitro* activities and mechanisms of action of anti-cancer molecules from African medicinal plants: a systematic review. *Am J Cancer Res*, 2024. 14(3): p. 1376–1401.
32. Imtiyaz, K., et al., Polydatin: A natural compound with multifaceted anticancer properties. *Journal of Traditional and Complementary Medicine*, 2025. 15(5): p. 447–466.
33. Mukherjee, S., et al., Therapeutic effect of targeted antioxidant natural products. *Discover Nano*, 2024. 19(1): p. 144.
34. Sadowska-Bartos, I. and G. Bartosz, Evaluation of The Antioxidant Capacity of Food Products: Methods, Applications and Limitations. *Processes*, 2022. 10(10): p. 2031.
35. Pan, S.Y., et al., Tea and tea drinking: China's outstanding contributions to the mankind. *Chin Med*, 2022. 17(1): p. 27.
36. Tao, L., et al., Association of Drinking Herbal Tea with Activities of Daily Living among Elderly: A Latent Class Analysis. *Nutrients*, 2023. 15(12).
37. Baek, G.-H., et al., Determination of methylxanthine contents and risk characterisation for various types of tea in Korea. *Food Control*, 2022. 132: p. 108543.
38. Rai, M.K., Start codon targeted (SCoT) polymorphism marker in plant genome analysis: current status and prospects. *Planta*, 2023. 257(2): p. 34.
39. Jedrzejczyk, I., Genome size and SCoT markers as tools for identification and genetic diversity assessment in *Echinacea* genus. *Industrial Crops and Products*, 2020. 144: p. 112055.
40. Chunarkar-Patil, P., et al., Anticancer Drug Discovery Based on Natural Products: From Computational Approaches to Clinical Studies. *Biomedicines*, 2024. 12(1): p. 201.
41. Maluleka, M.M., et al., Synthesis, structural characterization, and quantum chemical study of the 7-acetyl-5-nitrobenzofurans as anticancer agents with antioxidant properties. *Journal of Molecular Structure*, 2024. 1311: p. 138398.
42. Chen, B.K., et al., Improvement of *Echinacea purpurea* and *Ganoderma lucidum* Extracts with Cell Model on Influenza A/B Infection. *Molecules*, 2024. 29(15): p. 3609.
43. Azar Sadeghi, F.M., Farzaneh Sabouni, and Forough Sanjarian, Effect of *Echinacea purpurea*'s Extract on Gastric Cancer Cell (AGS) Growth, Migration, Colony Formation, Tube Formation, Apoptosis, and VEGF-A Gene Expression. *Systematic Reviews in Pharmacy*, 2022. 13(12): p. 870–881.

Supplementary Tables

Supplementary Table 1. The mean, variance, observations, correlation, hypothesized mean, difference, df, *t*-stat, *P*-value ($T \leq t$) one-tail, *t* Critical one-tail, *P*-value ($T \leq t$) two-tail, and *t* critical two-tail resulted from the *t*-test of the three cell lines' results

t-Test: viability and inhibitory for Means						
	Viability %	Inhibitory %	Viability %	Inhibitory %	Viability %	Viability %
	HCT-116 cell line		HeLa cell line		MCF-7 cell line	
Mean	87.92538462	12.07461538	92.23769	92.23769	89.41307692	10.58692308
Variance	398.2297936	398.2297936	171.0255	171.0255	339.2639397	339.2639397
Observations	13	13	13	13	13	13
Pearson correlation	-1		-1		-1	
Hypothesized mean difference	0		0		0	
df	12		12		12	
<i>t</i> Stat	6.852275183		11.64505		7.715128547	
<i>P</i> ($T \leq t$) one-tail	8.83253E-06		3.38E-08		2.71954E-06	
<i>t</i> Critical one-tail	1.782287556		1.782288		1.782287556	
<i>P</i> ($T \leq t$) two-tail	1.76651E-05		6.75E-08		5.43908E-06	
<i>t</i> Critical two-tail	2.17881283		2.178813		2.17881283	

Supplementary Table 2. The mean raw data of the viability, inhibition ratio, and S.D. ($\pm$) of HCT-116, HeLa, and MCF-7 cell lines

Sample conc. ($\mu\text{g/ml}$)	Viability %	Inhibitory %	S.D. ($\pm$)	Viability %	Inhibitory %	S.D. ($\pm$)	Viability %	Inhibitory %	S.D. ($\pm$)
	HCT-116 cell line			HeLa cell line			MCF-7 cell line		
8000	43.28	56.72	3.16	63.74	36.26	2.12	46.21	53.79	3.45
4000	56.43	43.57	2.71	69.28	30.72	1.64	59.43	40.57	1.91
2000	68.92	31.08	1.47	80.37	19.63	1.25	71.95	28.05	2.07
1000	75.69	24.31	2.15	86.16	13.84	1.22	85.82	14.18	1.34
500	98.71	1.29	0.83	99.54	0.46	0.38	98.96	1.04	0.82
250	100	0		100	0		100	0	
125	100	0		100	0		100	0	
62.5	100	0		100	0		100	0	
31.25	100	0		100	0		100	0	
15.6	100	0		100	0		100	0	
7.8	100	0		100	0		100	0	
3.9	100	0		100	0		100	0	
0	100	0		100	0		100	0	

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