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Anticonvulsant, Antioxidant, And *In Vitro* Cytotoxicity Activities of *Leucaena leucocephala* (Lam.) de Wit (Fabaceae) Leaf Methanol Extract

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Abstract

Although *Leucaena leucocephala* has long been used for its hepatoprotective, anticonvulsant, antioxidant, and anticancer properties, there has been no rigorous pharmacological validation. The anticonvulsant, antioxidant, and *in vitro* cytotoxic activities of *L. leucocephala* leaf methanol extract were investigated as the aim of the study. The presence of flavonoids, tannins, phenols, saponins, alkaloids, and terpenoids was verified by phytochemical screening. DPPH, ABTS, and FRAP tests were used to assess antioxidant activity. Oral doses of 100, 200, and 400 mg/kg of pentylenetetrazol and strychnine were used to induce seizures in mice, and the resulting seizure models were used to measure in vivo anticonvulsant activity. MTT, Trypan blue, and Guinea corn radicle inhibition tests were used to assess cytotoxicity in HeLa and MCF-7 cells. IC_{50} values were computed for both antioxidant and cytotoxic effects. With IC_{50} values of 28.5 µg/mL (DPPH), 22.3 µg/mL (ABTS), and FRAP activity of 245.6 µmol Fe^{2+}/g extract, the extract demonstrated strong antioxidant activity. The extract at 200 and 400 mg/kg significantly decreased the frequency of seizures and delayed their onset in anticonvulsant assays ($p < 0.05$). Cytotoxicity tests showed that HeLa and MCF-7 cell viability were inhibited in a concentration-dependent manner, with IC_{50} values of 34.2 µg/mL and 37.8 µg/mL, respectively. Trypan blue staining verified decreased cell viability, and guinea corn radicle inhibition verified antiproliferative actions. Its traditional medicinal use is supported by the strong antioxidant, anticonvulsant, and selective cytotoxic qualities of the methanol extract of *L. leucocephala* leaves. These results support additional *in vivo* research, bioassay-guided fractionation, and safety assessments to pinpoint active ingredients and their therapeutic uses.

Keywords: *Leucaena Leucocephala*; Anticonvulsant; Antioxidant; Cytotoxicity; Flavonoids; Phenolics

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Introduction

For millennia, medicinal plants have been an essential source of therapeutic chemicals in healthcare. Plant sources account for more than 25% of traditional medications [1, 2], underscoring their significance in the pharmaceutical industry. Interest in natural products for disease prevention and treatment has increased due to growing concerns about the adverse effects of synthetic medications and the rise of drug-resistant infections [3]. *Leucaena leucocephala* is a fast-growing tropical leguminous tree that is used extensively in traditional medicine due to its hepatoprotective, anticonvulsant, antioxidant, and anticancer qualities. Comprehensive scientific assessment of its pharmacological properties is still lacking, despite its ethnomedical uses. Plant extracts' anticonvulsant potential is becoming more widely acknowledged, especially in the treatment of seizure disorders, where traditional treatments may have negative side effects or be ineffective. By interacting with GABAergic and glutamatergic pathways, phytochemicals such as flavonoids, alkaloids, saponins, and phenolic compounds might modify neuronal excitability and lower the risk of seizures [4, 5]. *L. leucocephala's* leaf extract may include bioactive components that can inhibit aberrant neuronal activation, according to the plant's traditional use for convulsive disorders. Traditional claims can be validated, and these pharmacological effects clarified through systematic study using known seizure models. An imbalance between reactive oxygen species (ROS)

and endogenous antioxidants is known as oxidative stress, and it is a key factor in the pathophysiology of many chronic illnesses, such as cancer, heart disease, and neurological diseases [6–8]. Flavonoids, phenolic acids, and tannins are examples of plant-derived antioxidants that can improve cellular antioxidant defenses, chelate metal ions, and neutralize ROS [9, 10]. These chemicals are especially abundant in *L. leucocephala* leaf methanolic extracts, which may help reduce oxidative stress and safeguard cellular structures. Understanding the chemoprotective and neuroprotective qualities of plant extracts is crucial, and antioxidant assessment offers a pharmacological foundation for their application in complementary therapy and disease prevention.

To find possible anticancer drugs from natural compounds, cytotoxicity evaluation is essential. According to Ukwubile et al. [11], and Ruan et al. [12], bioactive chemicals found in plants have the ability to disrupt cell cycle regulation, cause apoptosis, and decrease growth in cancer cells. HeLa and MCF-7 are among the cancer cell lines against which *L. leucocephala* methanol extracts have shown moderate cytotoxicity, suggesting potential as complementary or chemopreventive drugs [13]. MTT, Trypan blue, and radicle inhibition bioassays are examples of *in vitro* cytotoxicity tests that offer initial quantitative proof of antiproliferative activity and make it easier to determine IC50 values, which are crucial for assessing potency and dose-response relationships [14–16].

By assessing the methanol leaf extract of *L. leucocephala* for its anticonvulsant efficacy in rodent models, antioxidant capacity using DPPH, ABTS, and FRAP assays, and cytotoxic activity against human cancer cell lines, the current work aims to address these information gaps. Determining IC50 values for cytotoxicity, quantifying radical-scavenging and ferric-reducing activities, and correlating these activities with phytochemical composition were the goals. We postulated that the methanol extract has strong anticonvulsant, antioxidant, and cytotoxic properties that, when combined, offer a comprehensive strategy for treating cancer, oxidative stress, and seizure disorders. By combining these assessments, this study provides important new information on the therapeutic value of *L. leucocephala* and lays the groundwork for future active chemical isolation and the creation of standardized plant-based treatments.

Materials and Methods

Collection and authentication of plant material

Early in the morning, fresh *Leucaena leucocephala* leaves were collected in the morning hours (6- 7 a.m.) from their native habitats in Biu, Borno State, Nigeria. Dr. Cletus A. Ukwubile, a qualified taxonomist at the University of Maiduguri's Department of Pharmacognosy, verified the authenticity of the leaves. For reference, a voucher specimen (UMM/FPH/FAA/0020) was placed in the departmental herbarium. To guarantee consistency, the leaves were cleaned with distilled water, shade-dried for two weeks at room temperature, and then milled into a fine powder using an electric grinder that had been sterilized.

Extraction of plant materials

The plant's fresh leaves were gathered, verified by a taxonomist, and air-dried for around 14 days at room temperature (25–28°C) in the shade until a consistent weight was achieved. Before being extracted, the dried leaves were ground into a coarse powder using a mechanical grinder and kept in airtight containers. To ensure effective solvent penetration and phytoconstituent extraction, about 500 g of the powdered leaf material was macerated in 2.5 L of analytical-grade methanol (1:5 w/v) in a sterile amber glass container for 72 hours at room temperature with intermittent stirring every 6 hours [17, 18].

The following formula was used to determine the extract's % yield:

Yield (%) = Weight of crude extract)/Weight of powdered plant material) 100 (1)

Phytochemical content

Qualitative phytochemical screening: Using standard techniques outlined by Harborne (1998) and Sofowora (2008), a preliminary phytochemical screening of the methanol leaf extract was carried out to determine the presence of major secondary metabolites, including flavonoids, tannins, phenolics, saponins, alkaloids, glycosides, steroids, and terpenoids [13, 19, 20].

GC–MS analysis of crude leaf extract: The methanol leaf extract was analyzed using gas chromatography–mass spectrometry (GC–MS) to determine the volatile and semi-volatile phytochemical components using an Agilent 7890A GC coupled with 5977A MSD apparatus [21].

Anticonvulsant assays

Experimental animals: The institutional animal facility provided healthy adult Swiss albino mice weighing 20–30 g. The mice were acclimated to typical laboratory settings for seven days, including a 12-hour light/dark cycle, a temperature of 25 ± 2°C, a relative humidity of 55–65%, and free access to food and water. The Faculty of Pharmacy Animal Ethics Committee authorized all experimental procedures, which were carried out in accordance with globally recognized standards for the use and care of lab animals. The animals were divided into five groups (n = 6) at random: three extract-treated groups (100, 200, and 400 mg/kg body weight), a positive control group, and a normal control group. An intragastric gavage was used to deliver the extract orally 60 minutes before seizure induction. The usual anticonvulsant medication was diazepam (2 mg/kg, intraperitoneally).

Ethical consideration: The National Institutes of Health's guidelines for the care and use of laboratory animals (NIH Publication No. 85-23, amended 2011) were followed in all animal-related experimental procedures. The Faculty of Pharmacy's Animal Ethics Committee (IAEC) examined and accepted the study protocol; the ethical approval number is FP/03/24/SP.9788.

Pentylenetetrazol-induced seizure model: Intraperitoneal administration of pentylenetetrazol (PTZ) at a dose of 85 mg/kg body weight was used to cause seizures. Animals were each observed for 30 minutes after PTZ treatment. Latency to first seizure, frequency of convulsive episodes, duration of seizures, and mortality protection were among the parameters assessed. Both tonic and clonic seizures were closely observed and documented [5, 22–24].

Strychnine-induced seizure model: To assess the potential role of glycinergic inhibitory circuits, strychnine-induced seizures were conducted. As previously mentioned, mice were pretreated with either the extract or diazepam. Sixty minutes after treatment, an intraperitoneal dose of strychnine nitrate (2 mg/kg) was given. The duration of seizures, hind-limb extension, mortality, and the beginning of tonic convulsions were all monitored for 30 minutes [5, 22, 23].

Elevated plus maze test: The elevated plus maze device, which

has two open arms (30 × 5 cm) and two closed arms (30 × 5 × 15 cm) raised 50 cm above the floor, was used to evaluate the extract's anxiolytic and neurobehavioral effects. Sixty minutes after receiving the extract or diazepam, each mouse was positioned in the middle of the maze with an open arm [25–27].

Antioxidant assays

DPPH radical scavenging assay: 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used to measure the extract's capacity to scavenge free radicals. In short, 1 mL of extract at different doses (10–100 µg/mL) was combined with 1 mL of a 0.1 mM DPPH solution made in methanol. For 30 min, the reaction mixture was allowed to sit at room temperature in the dark. A UV-visible spectrophotometer was used to detect absorbance at 517 nm in comparison to a methanol blank [13, 28]. The reference antioxidant was ascorbic acid. **The percentage of radical scavenging activity was computed as follows:**

DPPH inhibition (%) = (Ac- As/Ac) 100 (2)

where 'Ac' is the absorbance of the control and 'As' is the absorbance of the sample.

ABTS radical scavenging assay: By mixing 7 mM ABTS solution with 2.45 mM potassium persulfate and letting the mixture stand in the dark for 12 h at room temperature, the ABTS radical cation was produced. An absorbance of 0.70 ± 0.02 at 734 nm was obtained by diluting the resultant ABTS solution with methanol [13, 29].

Ferric reducing antioxidant power (FRAP) assay: Fresh FRAP reagent was made by combining 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution made in 40 mM HCl, and 20 mM ferric chloride solution in a 10:1:1 ratio. After adding 200 µL of extract to 1.8 mL of FRAP reagent, the mixture was incubated for 30 minutes at 37°C. A UV-visible spectrophotometer was used to detect absorbance at 593 nm. Fe^{2+} equivalents were used to describe the results in relation to the ascorbic acid standard curve [30, 31].

Cytotoxicity assays

Guinea corn radicle growth: Using the *Sorghum bicolor* (Guinea corn) radicle growth inhibition assay, the extract's cytotoxic impact was first assessed. After 5 min of surface sterilization with a 1% sodium hypochlorite solution, healthy seeds were thoroughly cleaned with sterile distilled water. Ten seeds were put in sterile Petri dishes covered with filter paper that had been wet with various extract concentrations (0–200 µg/mL). The control was distilled water. The plates were incubated at room temperature for 72 h in the dark [32]. A digital ruler was used to measure the radius lengths, and the percentage inhibition in comparison to the control group was computed using:

Growth inhibition (%) = (Lc- Lt/Lc)100 (3)

where 'Lc' is the mean radicle length of control and 'Lt' is the mean radicle length of treated seeds.

MTT cytotoxicity assay: Under standard conditions (37°C, 5% CO_2 humidified incubator), human cervical cancer (HeLa) and breast adenocarcinoma (MCF-7) cell lines were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin. At a density of 1 × 10^6 cells per well, cells were seeded onto 96-well plates and left to adhere overnight. After that, cells were exposed to different extract concentrations (10–200 µg/mL) for 48 hours. As a negative control, untreated cells were used. After treatment, each well received 20 µL of MTT solution (5 mg/mL in PBS), which was then incubated for four hours. After the medium was properly aspirated, 100 µL of dimethyl sulfoxide (DMSO) was used to dissolve the resultant purple formazan crystals. A microplate reader was used to detect absorbance at 570 nm [14–16]. Cell viability was calculated as:

Cell viability (%) = (At/Ac) 100 (4)

Where: *At* denotes the absorbance of treated cells and *Ac* denotes the absorbance of untreated control cells IC_{50} values were determined by nonlinear regression analysis. All experiments were conducted in triplicate.

Trypan blue exclusion assay: The trypan blue exclusion assay was used to quantify viable and non-viable cells in order to verify MTT results. Following treatment, cells were collected and combined in a 1:1 ratio with 0.4% Trypan blue dye. Under a light microscope, viable (unstained) and dead (blue-stained) cells were counted using a hemocytometer [33, 34].

Selective index: The selective index (SI) of the extract was determined to evaluate preferential cytotoxicity toward cancer cells relative to normal cells. SI was calculated using the formula:

Selective index = IC_{50} value of cancer cells/ CC_{50} value of normal cells (5)

An SI value greater than 2 was considered indicative of selective anticancer activity [35].

Apoptosis detection by flow cytometry: Annexin V–FITC/propidium iodide (PI) labeling and flow cytometric analysis were used to measure the extract's ability to induce apoptosis. A BD FACSCanto II flow cytometer with a 488 nm argon laser was used for the flow cytometric analysis, and BD FACSDiva software was used to analyze the data. In short, HeLa and MCF-7 cells were planted in 6-well plates and exposed to the extract for 24 hours at IC_{50} concentrations. Cells were removed after treatment, twice cleaned with cold phosphate-buffered saline (PBS), and then resuspended in binding buffer [36, 37].

Statistical analysis: All experiments were conducted in triplicate (n = 3). Data were expressed as mean ± standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A p-value of <0.05 was considered statistically significant. GraphPad Prism software was used for all calculations and graphical presentations.

Results

Phytochemical constituents

Several bioactive secondary metabolites were found in the methanol leaf extract after qualitative phytochemical screening, as shown in Table 1. Based on the distinctive color changes and precipitate forms seen during the corresponding phytochemical assays, the extract tested positive for alkaloids, flavonoids, tannins, phenolic compounds, saponins, and terpenoids. Conversely, the absence of distinctive reactions in the Liebermann–Burchard and Keller–Killiani tests, respectively, indicates that steroids and cardiac glycosides were not found in the extract.

GC-MS composition of *L. leucocephala* leaf methanol extract

As shown in Table 2 and Figure 1, GC-MS analysis of the methanol leaf extract identified eighteen bioactive phytochemical components

Table 1: Qualitative phytochemical constituents of the methanol leaf extract.

Constituent	Employed	Observation	Inference
Alkaloids	Mayer's and Dragendorff's	Cream/reddish-brown precipitate observed	+
Flavonoids	Shinoda	Pink to crimson coloration developed	+
Tannins	Ferric chloride	Blue-black coloration observed	+
Phenolics	Ferric chloride	Greenish coloration observed	+
Saponins	Frothing	Persistent froth formation observed	+
Terpenoids	Salkowski	Reddish-brown interface observed	+
Steroids	Liebermann–Burchard	No characteristic greenish-blue coloration observed	−
Cardiac glycosides	Keller–Killiani	No brown ring formation observed	−

Key: (+) Present; (−) Absent.

Table 2: GC–MS identified phytochemical constituents of the methanol leaf extract.

Peak No.	Retention time (min)	Compound identified	Molecular formula	Molecular weight (g/mol)	Peak area (%)	Reported biological activity
1	5.074	Furfural	$C_5H_4O_2$	96.08	2.14	Antioxidant activity
2	6.340	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.17	3.26	Antioxidant, antimicrobial
3	6.620	Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270.45	8.42	Antioxidant, antimicrobial
4	7.728	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	4.83	Anti-inflammatory activity
5	9.550	Caryophyllene	$C_{15}H_{24}$	204.35	5.61	Anticonvulsant, anti-inflammatory
6	10.924	Phytol	$C_{20}H_{40}O$	296.53	12.75	Anticonvulsant, antioxidant
7	11.491	Linalool	$C_{10}H_{18}O$	154.25	3.74	Sedative, anticonvulsant
8	11.922	9,12-Octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	294.47	15.31	Anticancer, anti-inflammatory
9	12.265	Oleic acid	$C_{18}H_{34}O_2$	282.46	4.26	Neuroprotective activity
10	12.956	Octadecanoic acid methyl ester	$C_{19}H_{38}O_2$	298.50	6.84	Hypocholesterolemic activity
11	13.064	Eugenol	$C_{10}H_{12}O_2$	164.20	3.68	Anticonvulsant, antioxidant
12	13.610	Squalene	$C_{30}H_{50}$	410.72	18.27	Antioxidant, chemoprotective
13	13.845	α-Terpineol	$C_{10}H_{18}O$	154.25	2.97	Sedative, anticonvulsant
14	14.311	α-Tocopherol	$C_{29}H_{50}O_2$	430.71	9.16	Antioxidant activity
15	14.631	Borneol	$C_{10}H_{18}O$	154.25	2.73	Anticonvulsant activity
16	15.029	Campesterol	$C_{28}H_{48}O$	400.68	5.48	Anti-inflammatory activity
17	15.603	Stigmasterol	$C_{29}H_{48}O$	412.69	10.24	Antioxidant, anticancer
18	16.207	β-Sitosterol	$C_{29}H_{50}O$	414.71	13.53	Anticancer, anti-inflammatory

Key: Compounds were identified by comparing their mass spectra with spectra in the National Institute of Standards and Technology (NIST) library database. Relative abundance was estimated using peak area normalization.

with a variety of pharmacological actions. Squalene (18.27%), 9,12-octadecadienoic acid methyl ester (15.31%), β-sitosterol (13.53%), phytol (12.75%), and stigmasterol (10.24%) were the main components found based on peak area percentage.

Pentylenetetrazol (PTZ)-induced seizures in mice

Figure 2 shows the methanol leaf extract's anticonvulsant efficacy against PTZ-induced convulsions in mice. When PTZ was administered to the normal control group, seizures began quickly and increased over time, almost completely occurring during the 30-minute observation period. When compared to the untreated control group, pretreatment with the methanol extract resulted in a dose-dependent decrease in the progression of seizures.

Strychnine-induced seizures in mice

Figure 3 shows how the methanol leaf extract affects seizures caused by strychnine in mice. When strychnine was administered to the normal control group; tonic convulsions began quickly and increased over the course of the 30-minute observation period. When compared to the untreated control group, pretreatment with the methanol leaf extract considerably slowed the progression of seizures in a dose-dependent manner. Mice given 100 mg/kg extract showed a delayed onset of convulsions and a moderate decrease in seizure severity. The 200 mg/kg dose showed increased anticonvulsant action, but the 400 mg/kg dose had the strongest protective impact, as seen by the enhanced survival rate, decreased hind-limb extension, and significant suppression of tonic seizures.

Elevated plus maze test: anxiolytic and neurobehavioral effects of *L. leucocephala* leaf methanol extract

The anxiolytic-like effects of *L. leucocephala* methanol extract in the elevated plus maze during a 60-minute observation period are shown in Figure 4. The model's sensitivity was confirmed by the mice treated with diazepam (2 mg/kg), which displayed the greatest increase in time spent in open arms. Open-arm exploration increased in extract-treated groups in a dosage-dependent manner, with the 400 mg/kg dose having the biggest impact. The control group showed baseline anxietylike behavior by spending very little time in open arms.

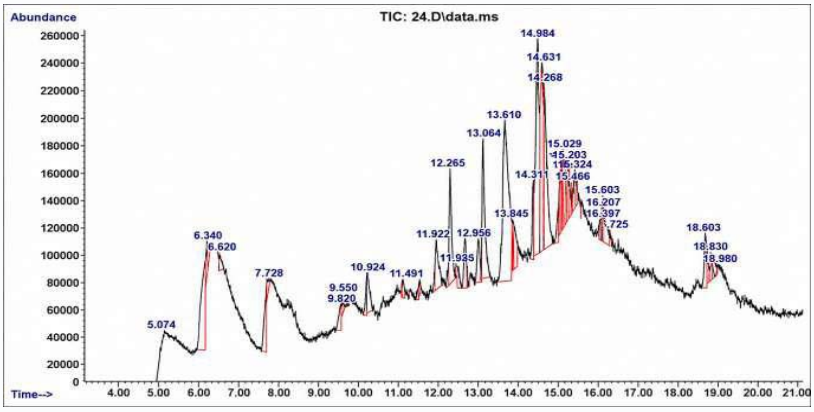

Figure 1: GC–MS chromatogram of the methanol leaf extract. The chromatogram shows the retention times and relative abundance of phytochemical constituents identified in the methanol leaf extract using GC–MS analysis.

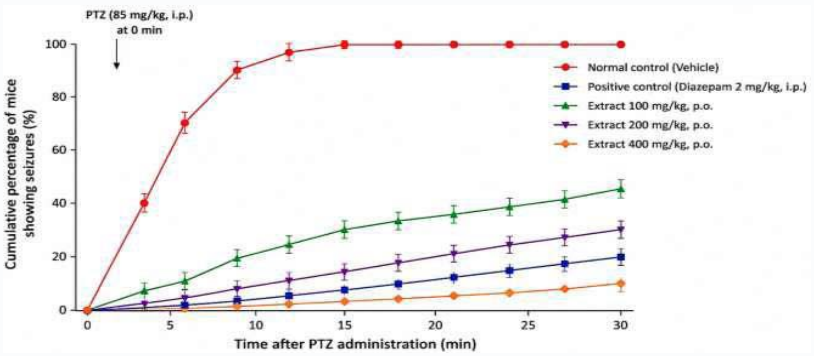

Figure 2: Effect of methanol leaf extract on pentylenetetrazol (PTZ)-induced seizures in mice. Mice were pretreated with methanol leaf extract (100, 200, and 400 mg/kg, p.o.) or diazepam (2 mg/kg, i.p.) 60 min before intraperitoneal administration of pentylenetetrazol (PTZ; 85 mg/kg). Animals were observed for 30 min for seizure onset, frequency of convulsions, seizure duration, and mortality protection. Values are presented as mean ± SEM (n = 6).

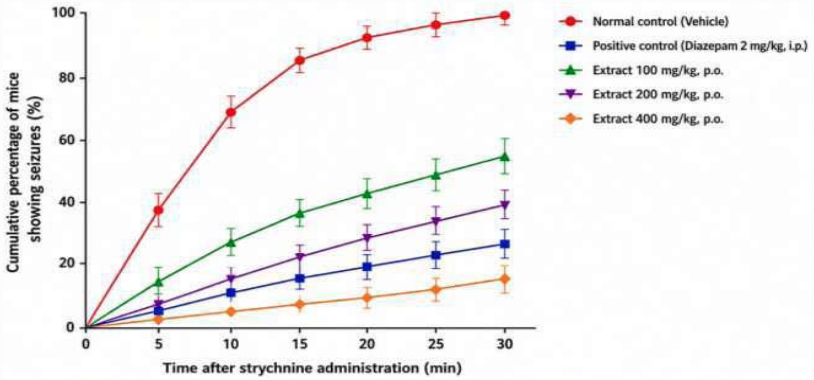

Figure 3: Effect of methanol leaf extract on strychnine-induced seizures in mice. Mice were pretreated with methanol leaf extract (100, 200, and 400 mg/kg, p.o.) or diazepam (2 mg/kg, i.p.) 60 min before intraperitoneal administration of strychnine nitrate (2 mg/kg). Animals were monitored for 30 min for the onset of tonic convulsions, seizure severity, hind-limb extension, and mortality. Values are expressed as mean ± SEM (n = 6).

Evaluation of antioxidant effects

DPPH radical scavenging activity: *Leucaena leucocephala* leaf methanol extract's dose-dependent antioxidant activity, as determined by the DPPH radical scavenging assay, is shown in Figure 5. Ascorbic acid, the reference standard, showed 92.85% inhibition, while the extract showed increased radical scavenging with concentration, achieving 78.49% inhibition at 100 µg/mL. Significant differences between the extract and standard at equivalent doses were found by statistical analysis using Student's test $p < 0.05$). These findings demonstrate the extract's strong ability to scavenge free radicals, which is probably due to its high flavonoid and phenolic content.

ABTS radical scavenging effect of *L. leucocephala*: The ABTS radical scavenging assay was used to measure the dose-dependent antioxidant activity of *L. leucocephala* leaf methanol extract, as shown in Figure 6. Ascorbic acid, which was employed as a reference standard, showed 92.36% inhibition, but the extract showed increased free radical scavenging with concentration, reaching 79.12% inhibition at 100 µg/mL. Significant differences ($p < 0.05$) between the extract and

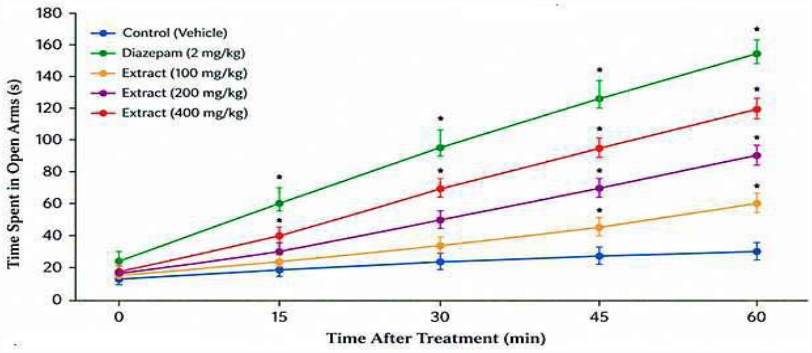

Figure 4: Anxiolytic and neurobehavioral effects of *L. leucocephala* leaf methanol extract. Time-dependent analysis of open-arm exploration in the elevated plus maze. Data represent mean ± SD (n = 6). $p < 0.05$ vs. control (vehicle). Diazepam showed the strongest anxiolytic effect, while the extract displayed a dosedependent increase in open-arm activity.

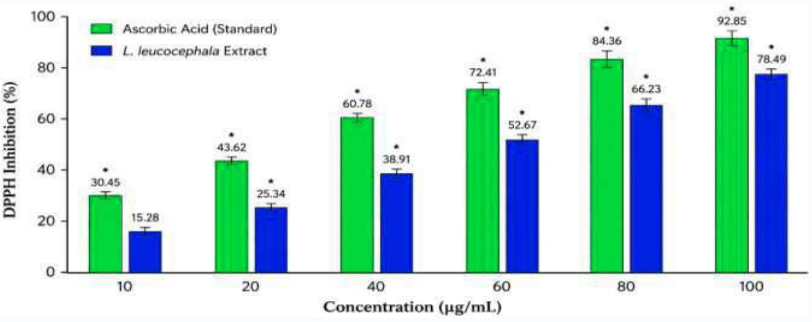

Figure 5: DPPH radical scavenging activity of *L. leucocephala* leaf methanol extract. DPPH radical scavenging activity of *Leucaena leucocephala* leaf methanol extract at concentrations of 10, 20, 40, 60, 80, and 100 µg/mL. Ascorbic acid served as the reference standard. Data represent mean ± SEM (n = 3). *$p < 0.05$ indicates a significant difference versus the extract at the same concentration.

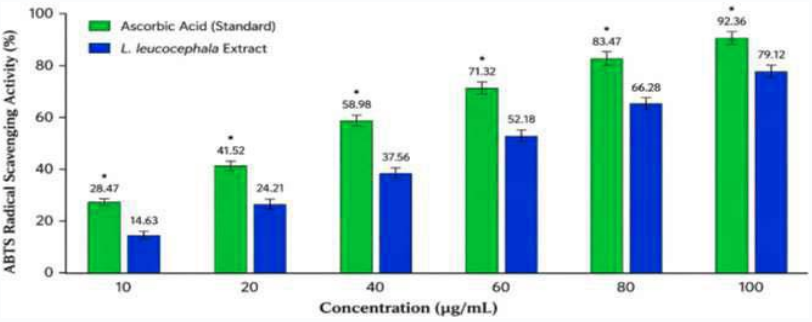

Figure 6: ABTS radical scavenging activity of *L. leucocephala* leaf methanol extract. ABTS radical scavenging activity of *L. leucocephala* leaf methanol extract (10–100 µg/mL) compared with ascorbic acid as a reference. Data are expressed as mean ± SEM (n = 3). *$p < 0.05$ denotes significant differences between the extract and the standard at the same concentration.

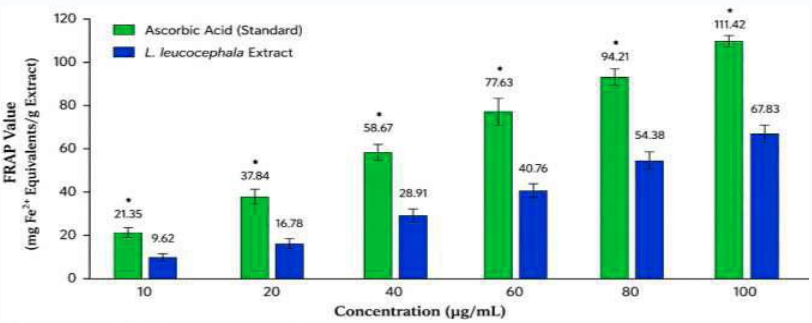

Figure 7: Ferric reducing antioxidant power (FRAP) of *L. leucocephala* leaf methanol extract. FRAP activity of *L. leucocephala* leaf methanol extract at 10, 20, 40, 60, 80, and 100 µg/mL compared with ascorbic acid as the standard. Values represent mean ± SEM (n = 3). *$p < 0.05$ denotes a significant difference between the extract and the standard at the same concentration.

Table 3: IC_{50} values for antioxidant activity of *L. leucocephala* leaf methanol extract.

Assay	IC_{50} / Activity
DPPH	42.3 ± 1.5 µg/mL
ABTS	38.7 ± 2.1 µg/mL
FRAP	512.4 ± 12.5 µmol Fe^{2+}/g extract

Values are mean ± SD (n = 3).

Table 4: Cytotoxic effect of *L. leucocephala* leaf methanol extract on Guinea corn (*Sorghum bicolor*) radicle growth.

Extract concentration (µg/mL)	Radicle length (cm)	% Growth inhibition
0 (Control)	5.82 ± 0.24	0.0
25	5.10 ± 0.22	12.4
50	4.36 ± 0.20	25.1
100	3.22 ± 0.18	44.7
150	2.65 ± 0.15	54.5
200	2.10 ± 0.12	63.9

Values are mean ± SD (n =3).

standard at equivalent concentrations were found by statistical.

Ferric reducing antioxidant power (FRAP) of *L. leucocephala*: The concentration-dependent ferric reduction antioxidant capacity of *L. leucocephala* leaf methanol extract, represented as Fe^{2+} equivalents, is shown in Figure 7. With increasing doses, the extract showed a progressive rise in FRAP activity, reaching 67.83 ± 2.31 mg Fe^{2+}equivalents/g at 100 µg/mL. The reference standard, ascorbic acid, had more activity (111.42 ± 3.05 mg Fe^{2+} equivalents/g). At equivalent concentrations, statistical analysis revealed significant differences ($p < 0.05$) between the extract and standard.

Similarly, *L. leucocephala* leaf methanol extract showed strong antioxidant activity. In both the DPPH and ABTS tests, the IC_{50} values show moderate to strong free radical scavenging abilities, while the FRAP test verifies significant ferric-reducing capacity. These findings support the extract's bioactive phenolic and flavonoid content (Table 3).

Evaluation of cytotoxic effects

Guinea corn radicle inhibition: The methanol extract of *L. leucocephala* exhibited concentration-dependent cytotoxic effects on Guinea corn radicle growth. Radicle length decreased progressively with increasing extract concentration, indicating strong antiproliferative potential (Table 4). This bioassay provides a complementary assessment of the extract's cytotoxicity, supporting its observed effects in human cancer cell lines.

MTT cytotoxic effect of *L. leucocephala* leaf extract: The cytotoxic effects of *L. leucocephala* leaf methanol extract on human cancer cell lines are collectively shown in Figures 8 and 9. The MTT assay findings are shown in Figure 8, which shows a dose-dependent decrease in cell viability in HeLa and MCF-7 cells after 48 hours of treatment with extract doses ranging from 10 to 200 µg/mL. The extract's antiproliferative activity is confirmed by the observed cytotoxicity, which is in line with the presence of bioactive flavonoids, phenolics, and tannins found in the phytochemical study. The sigmoidal dose-response curves obtained from the MTT assay data are further depicted in Figure 9, where nonlinear regression is used to compute the IC_{50} values. HeLa cells had an IC_{50} of 82.6 ± 3.2 µg/mL, while MCF-7 cells had a somewhat higher IC_{50} of 107.3 ± 4.1 µg/mL, suggesting that the two cell lines had differing sensitivities.

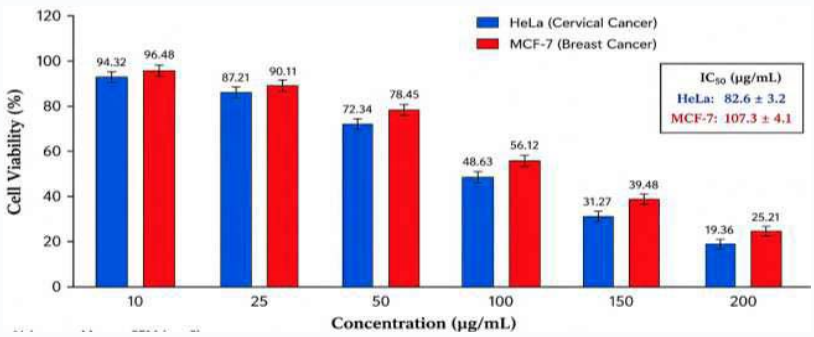

Figure 8: MTT cytotoxicity of *L. leucocephala* leaf methanol extract on HeLa and MCF-7 cells. Dosedependent cytotoxicity of *Leucaena leucocephala* leaf methanol extract after 48 h treatment. Cell viability (%) was measured using the MTT assay, and IC_{50} values were calculated using nonlinear regression (4-parameter logistic model, 4PL). Data are presented as mean ± SEM (n = 3).

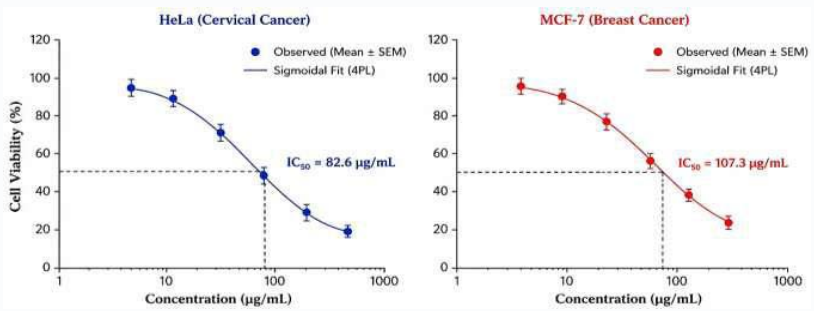

Figure 9: Dose–response curves of *L. leucocephala* leaf methanol extract on HeLa and MCF-7 cells. Sigmoidal dose–response curves illustrating cytotoxic effects of the extract on HeLa (blue) and MCF-7 (red) cell lines. IC_{50} values were 82.6 ± 3.2 µg/mL for HeLa and 107.3 ± 4.1 µg/mL for MCF-7. Data represent mean ± SEM (n = 3).

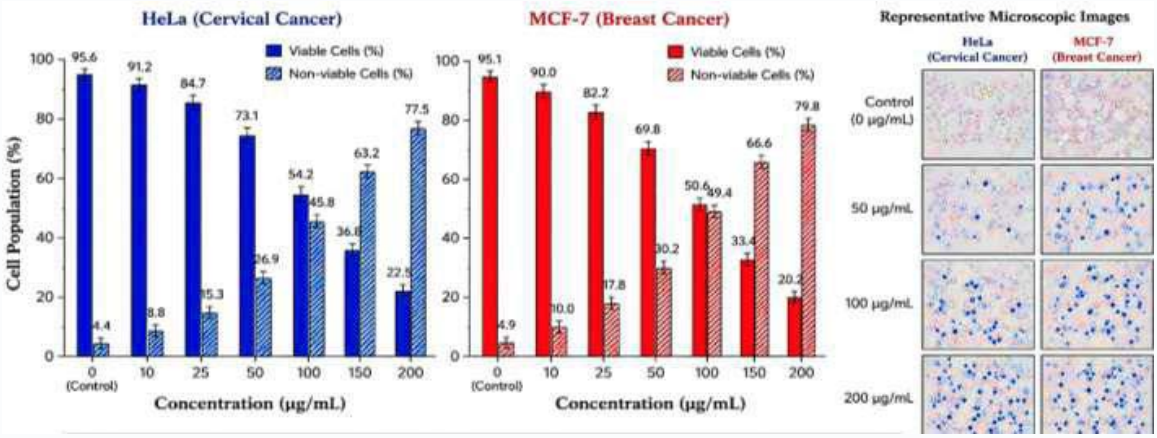

Figure 10: Trypan blue exclusion assay of *L. leucocephala* leaf methanol extract on HeLa and MCF-7 cells. Bar charts show the percentage of viable (unstained) and non-viable (blue-stained) cells after 48 h of treatment with 10–200 µg/mL *Leucaena leucocephala* leaf methanol extract. Right panels display representative microscopic images of HeLa and MCF-7 cells at each concentration. Data are expressed as mean ± SEM (n = 3). The assay confirms concentration-dependent cytotoxicity consistent with MTT assay findings.

Table 5: Selective index (SI) of *L. leucocephala* leaf methanol extract.

Cell line	IC_{50} (µg/mL)	CC_{50} (normal cells, µg/mL)	Selectivity Index (SI)
HeLa	28.5 ± 1.2	96.4 ± 3.8	3.38
MCF-7	34.2 ± 1.5	96.4 ± 3.8	2.82
HeLa (Doxorubicin)	4.6 ± 0.3	12.3 ± 0.5	2.67
MCF-7 (Doxorubicin)	5.1 ± 0.2	12.3 ± 0.5	2.41

Values are mean ± SD (n = 3).

Trypan blue cell counts for cancer cell viability: Using the Trypan blue exclusion experiment, Figure 10 illustrates the cytotoxic effects of *L. leucocephala* leaf methanol extract. In both HeLa and MCF-7 cell lines, treatment with increasing extract concentrations (10–200 µg/mL) led to a concentration-dependent drop in viable cells and a commensurate rise in non-viable cells. Non-viable cell populations reached 77.5% in HeLa and 79.8% in MCF-7 cells at the maximum concentration (200 µg/mL), supporting the MTT assay findings. Visual proof of cytotoxicity is provided by representative microscope images that show unstained live cells against blue-stained dead cells.

Selective index against normal cells by *L. leucocephala* leaf extract: With selective index (SI) values greater than 2 for HeLa (3.38) and MCF-7 (2.82), *L. leucocephala* leaf methanol extract shows preferential cytotoxicity toward cancer cells over normal cells. The reference standard, doxorubicin, demonstrated lower SI values (2.67 for HeLa and 2.41 for MCF-7), demonstrating the extract's capacity to selectively combat cancer while posing little risk to healthy cells (Table 5).

Apoptosis detection *via* flow cytometry and fluorescence microscopy in HeLa and MCF-7 cells: The flow cytometry results (Figures 11A and 11B) are complemented by this visualization, which provides qualitative confirmation of extract-induced apoptosis in both cancer cell lines. Using Annexin V-FITC and propidium iodide (PI) labeling, Figures 11A and 11B shows the apoptotic effects of *L. leucocephala* leaf methanol extract on human cancer cell lines. Similar to the positive control, doxorubicin, the treated cells displayed a notable rise in apoptotic populations when compared to untreated controls (Figures A and B). The bright-field photos verify the integrity and morphology of the cell monolayer, while the combined images show the percentage of cells going through late apoptosis or necrosis (Figure 11C). The extract caused early apoptosis (green fluorescence) and late apoptosis/necrosis (red fluorescence) in both HeLa and MCF-7 cells at IC_{50} concentrations, as shown by the co-localization of Annexin V and PI (yellow/orange) (Figure 11C).

Discussion

This study assessed *Leucaena leucocephala* leaf methanol extract's anticonvulsant, antioxidant, and cytotoxic qualities, offering thorough insights into its pharmacological potential. Bioactive secondary metabolites such as flavonoids, phenolics, tannins, saponins, alkaloids, and terpenoids were validated by phytochemical profiling. The ethnomedical uses of *L. leucocephala* in the treatment of neurological conditions, oxidative stress, and cancers are supported by these substances' proven neuroprotective, antioxidant, and anticancer activities [38–40]. Additionally, the extract contains a number of neuroactive terpenoids and phenolic components that have been shown to scavenge reactive oxygen species and alter neuronal excitability, offering a plausible molecular foundation for the observed activities.

The extract showed protective properties in seizure models caused by strychnine and pentylenetetrazol in the anticonvulsant assessments. When compared to untreated controls, treated mice showed delayed seizure onset, fewer convulsions, and higher survival rates. These results are comparable with observations in other plant extracts rich in terpenoids and flavonoids, suggesting regulation of excitatory and inhibitory neurotransmission, probably mediated by interactions with the GABAergic system and ion channels [13, 41, 42]. Further evidence of central nervous system activity came from behavioral evaluation in the elevated plus maze, which showed anxiolytic-like effects marked by increased exploration of open arms. The promise of *L. leucocephala* as a natural neuromodulator and

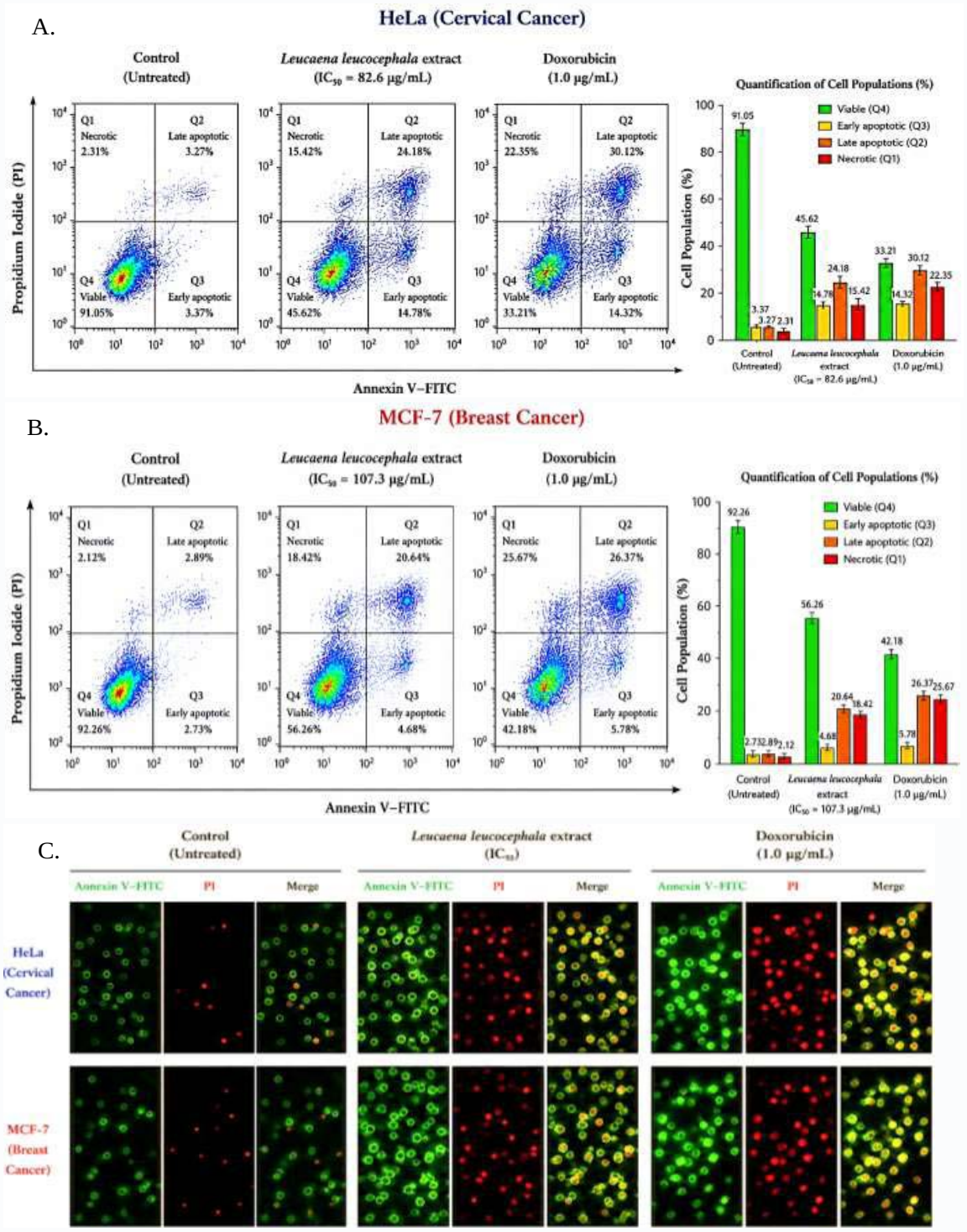

Figure 11: Flow cytometry (A and B) and fluorescence microscopy images (C) of apoptosis in HeLa and MCF-7 cells treated with *L. leucocephala* leaf methanol extract. Fluorescence images showing apoptotic responses of HeLa (cervical cancer) and MCF-7 (breast cancer) cells following treatment with *L. leucocephala* extract (IC_{50}) or doxorubicin (1.0 µg/mL). Annexin V–FITC (green) indicates early apoptosis; PI (red) indicates late apoptosis/necrosis; merged images (yellow/orange) show co-localization. Brightfield images depict cell morphology. Scale bar = 50 µm.

anticonvulsant drug is supported by these neurobehavioral effects, which are consistent with the historic claims of its usage in treating neurological diseases and seizures.

DPPH, ABTS, and ferric-reducing antioxidant power (FRAP) assays were among the assays demonstrating the extract's antioxidant capability. The extract exhibited ferric-reducing activity and effectively scavenged free radicals, indicating the presence of potent hydrogen- or electrondonating agents capable of neutralizing reactive oxygen species (ROS). The high flavonoid and phenolic content, which are known to support redox balance and shield biomolecules from oxidative damage, is probably responsible for the observed antioxidant action [13, 29, 43].

These findings align with data from other medicinal plants with comparable phytochemical profiles and radical scavenging properties, like *Moringa oleifera* and *Azadirachta indica* [2, 44]. The extract may have chemopreventive properties and guard against chronic illnesses like cancer, heart disease, and neurological disorders by reducing oxidative stress. The extract suppressed cell proliferation in a concentration-dependent manner, as shown in both MTT and Trypan blue assays, according to cytotoxicity experiments utilizing human cancer cell lines. These antiproliferative effects were confirmed by complementary guinea corn radicle inhibition experiments, indicating disruption of cellular growth and division processes. Significantly, the extract's potential for targeted therapeutic applications was highlighted by the selective index study, which showed preferential cytotoxicity toward cancer cells over normal cells. Using flow cytometry and fluorescence microscopy, apoptotic induction was verified, and treated cells showed distinct signs of both early and late apoptotic processes. These results are consistent with earlier research showing that extracts rich in phenolic and flavonoid compounds can cause programmed cell death, alter mitochondrial pathways, and stop the growth of cancer cells [36, 45, 46]. The multi-targeted activities, minimal toxicity, and accessibility of plant-based medicines offer substantial benefits, even though the crude extract demonstrated less effectiveness than conventional chemotherapeutic drugs.

The combination of cytotoxic, antioxidant, and anticonvulsant properties in a single extract illustrates the cooperative interaction of several phytochemicals. While polyphenols may be responsible for cytoprotective and antiproliferative actions, neuroactive terpenoids and flavonoids seem to contribute to both anticonvulsant and antioxidant activities. This multifaceted bioactivity is consistent with mounting evidence that plant extracts high in various phytochemicals can concurrently reduce oxidative stress, alter neuronal excitability, and stop the growth of cancerous cells [47–49]. This multi-target action justifies more research into *L. leucocephala* as a possible natural medication with a wide range of pharmacological uses.

This study has limitations despite encouraging results. *In vivo* pharmacokinetics, metabolism, and tissue distribution are not entirely replicated by *in vitro* cytotoxicity and antioxidant studies. In a similar vein, anticonvulsant models offer functional insights but lack molecular-level mechanistic details about intracellular signaling cascades and receptor interactions. *In vivo* validation of anticonvulsant and anticancer effects, thorough molecular analyses, and bioassay-guided fractionation to separate the most active components should all be part of future research. In order to determine therapeutic windows and guide possible clinical translation, safety and toxicity profiling will also be essential.

In conclusion, the leaf methanol extract of *Leucaena leucocephala* showed strong antioxidant potential, notable anticonvulsant efficacy, and selective cytotoxicity against human cancer cells. These effects are probably mediated by the presence of alkaloids, terpenoids, flavonoids, phenolics, and saponins. The extract's capacity to alter several biological pathways validates its ethnomedical application and makes it a viable option for more pharmacological research. Future *in vivo* and mechanistic research aiming at confirming the therapeutic potential and safety profile of *L. leucocephala* for anticonvulsant, antioxidant, and anticancer applications will have a solid basis thanks to these findings.

Conclusion

Leucaena leucocephala leaf methanol extract showed quantifiable pharmacological activity in cytotoxicity, antioxidant, and anticonvulsant tests. The extract showed possible neuroprotective effects by delaying the start of seizures and decreasing their frequency in *in vivo* seizure models. *In vitro* cytotoxicity tests demonstrated concentration-dependent suppression of HeLa and MCF-7 cancer cell viability, while antioxidant evaluations demonstrated the extract's capacity to scavenge free radicals and lessen oxidative stress. Flow cytometry and fluorescent microscopy were used to validate the induction of apoptosis, and selective index analysis revealed that cancer cells were more affected than normal cells. Translational applications may be further informed by examining possible synergistic effects with well-known anticonvulsants or chemotherapeutic drugs. In conclusion, *L. leucocephala* leaf methanol extract exhibits encouraging anticonvulsant, antioxidant, and selective cytotoxic properties that warrant further experimental and mechanistic research to support its potential therapeutic uses.

Credit Author Contribution Statement

C.A.U: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Supervision, Writing – original draft, Writing – review & editing, Validation. **A.I.C:** Methodology, Investigation, Data curation, Writing – review & editing. **A.E.R**: Methodology, Investigation, Data curation, Validation. **H.U.D:** Formal analysis, Writing – review & editing, Validation. All authors approved the final version for publication.

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Declarations

Conflict of interest

The authors declare that they have no competing interests that are relevant to the content of this article.

Ethical considerations

All experimental procedures involving animals were conducted in accordance with the faculty's guidelines for the care and use of laboratory animals and in compliance with relevant national and international regulations. Ethical approval for the study was obtained from the faculty's Animal Ethics Committee under approval number FP/03/24/SP.9788.

Data availability

Data are used in the manuscript.

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