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Toxicity, cytotoxicity, and anti-inflammatory activities of polyphenols from *Guiera senegalensis* J.F. Gmel. leaf extract

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Polyphenols are bioactive compounds with notable pharmacological properties, including anti-inflammatory, cytotoxic, and neuroprotective activities. This study investigated the anti-cancer, anti-inflammatory, and neuroprotective properties of polyphenols extracted from *Guiera senegalensis*. Phytochemical analyses confirmed high levels of phenolics and flavonoids, with catechins, quercetin, and kaempferol identified using FTIR, GC-MS, and NMR. Toxicity studies validated the safety of these polyphenols at therapeutic doses. Cytotoxicity assays revealed enhanced anti-cancer effects compared to doxorubicin, with reduced cell viability, colony formation, and migration in MCF-7 and OV7 cancer cell lines. Mechanistic insights showed ROS-induced mitochondrial dysfunction, caspase-3 activation, and G2/M cell cycle arrest, indicating apoptosis induction. The polyphenols also demonstrated significant anti-inflammatory effects, downregulating TNF-α, IL-1β, iNOS, and NF-κB expression. The neuroprotective activity was evidenced by increased levels of TH, TPH, DAT, SERT, and BDNF, alongside reduced Iba1 expression, suggesting mitigation of neuroinflammation and enhanced neurotransmitter function. These findings highlight the multi-targeted efficacy of *G. senegalensis* polyphenols in modulating apoptosis, inflammation, and neuroprotection, surpassing doxorubicin. Their dual anti-cancer and neuroprotective effects underscore their potential as safe therapeutic agents. Further research is warranted to explore their translational applications in integrative medicine for cancer and neuroinflammatory disorders.

Keywords: *Guiera senegalensis, polyphenols, cancer therapy, anti-inflammatory activity, neuroprotection, apoptosis*

Introduction

Cytotoxicity and inflammation are intricately linked processes that significantly influence human health, particularly in the context of cancer development and progression (Majdalawieh & Fayyad, 2016). Chronic inflammation, often triggered by infections, environmental toxins, or autoimmune diseases, plays a central role in promoting various stages of tumorigenesis, including initiation, promotion, and metastasis (Korniluk et al., 2017). Persistent inflammatory responses create a tumour-friendly microenvironment by inducing genetic mutations, activating oncogenic signalling pathways, stimulating angiogenesis, and suppressing the immune system's ability to detect and eliminate cancer cells (Uwishema et al., 2023). This pro-inflammatory milieu is exacerbated by the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which contribute to oxidative stress. These reactive species damage cellular macromolecules such as DNA, lipids, and proteins, further enhancing the risk of malignancy and fueling cancer progression (Sahu et al., 2023). The dual presence of oxidative stress and chronic inflammation not only fosters tumour growth but also complicates therapeutic intervention, highlighting the critical need for effective strategies to target both processes simultaneously.

Natural bioactive compounds, particularly polyphenols from medicinal plants, have emerged as promising agents for addressing this dual challenge. Polyphenols, a diverse group of plant-derived secondary metabolites characterized by their phenolic structures, possess a wide spectrum of biological activities, including potent antioxidant, anti-inflammatory, and anticancer effects (Bencheikh et al., 2022). These compounds neutralize ROS, inhibit pro-inflammatory signalling pathways, and induce apoptosis in cancer cells, disrupting the vicious cycle of inflammation and tumour growth. Polyphenols also offer the advantage of being safer and less toxic compared to conventional therapies, which are often associated with significant side effects (Aljutaily et al., 2023). By targeting both cytotoxicity in cancer cells and inflammation-related pathways, these natural compounds represent a promising avenue for therapeutic development. As such, the exploration of polyphenol-rich plants like *Guiera senegalensis* is vital for identifying novel agents that can effectively combat inflammation-associated cancers while minimizing adverse effects.
Inflammation, cancer, and neurotransmitter dysregulation are interlinked biological processes that contribute to disease progression and pathology (Ca et al., 2024). This inflammation can drive tumorigenesis by facilitating angiogenesis, immune evasion, and metastasis. Additionally, inflammation often impacts the central nervous system (CNS), leading to neuroinflammation, which can exacerbate cancer progression and impair neurotransmitter regulation (Koskela et al., 2021). Altered neurotransmitter signalling, particularly dopamine, serotonin, and norepinephrine, has been implicated in cancer-related fatigue, pain, and depression, affecting the quality of life in cancer patients (Murtala & Akindele, 2020). Plant-derived polyphenols, abundant in fruits, vegetables, and herbs, offer promising therapeutic potential due to their ability to target multiple disease pathways (Belew et al., 2021). These compounds exhibit cytotoxic effects on cancer cells by inducing oxidative stress and apoptosis. Polyphenols modulate key signalling pathways, such as NF-κB, MAPK, and caspase activation, leading to reduced cell viability and tumour growth. Furthermore, polyphenols are known to reduce inflammation by inhibiting pro-inflammatory cytokines and enzymes like COX-2 and iNOS, disrupting the inflammatory cascade. In the context of neuroinflammation, polyphenols can regulate neurotransmitter synthesis and release, promoting neuroprotection by reducing microglial activation and enhancing neuronal function (Bencheikh et al., 2022). Thus, polyphenols represent a multi-targeted therapeutic approach for managing both cancer and its associated neuroinflammatory and neurological impacts (Adefegha et al., 2018).

Guiera senegalensis, a medicinal plant native to sub-Saharan Africa, is highly regarded in traditional medicine for its diverse therapeutic applications (Abubakar et al., 2006). The plant is commonly used in the treatment of conditions such as fever, wounds, diarrhoea, respiratory infections, and inflammation (Abubakar et al., 2006). Its widespread use in ethnomedicine is supported by anecdotal evidence, but scientific validation of its pharmacological potential remains limited. Emerging studies suggest that *G. senegalensis* contains bioactive compounds, including alkaloids, flavonoids, tannins, and other polyphenols, which may contribute to its therapeutic effects (Elmalik et al., 2022). However, the precise bioactive constituents responsible for its anti-inflammatory and anticancer activities have yet to be fully elucidated. The present study addressed the knowledge gap by successfully isolating and characterizing the polyphenolic compounds present in the leaves of *G. senegalensis*. The cytotoxic and anti-inflammatory activities of these compounds were comprehensively evaluated through both *in vitro* and *in vivo* models. The cytotoxic effects of the isolated polyphenols were tested against MCF-7 (human breast adenocarcinoma) and OV7 (human ovarian cancer) cell lines, which are widely used in cancer research due to their relevance in studying hormone-dependent and hormone-independent cancers, respectively (Ukwubile et al., 2024). The results demonstrated significant cytotoxic activity, with the polyphenols exhibiting higher efficacy than standard drugs in reducing cell viability in both cell lines. This study provides a comprehensive understanding of the pharmacological actions of *G. senegalensis*, paving the way for its potential development into effective natural therapies for inflammation and cancer. With the increasing global burden of these diseases, particularly in resource-limited settings, the therapeutic application of indigenous medicinal plants like *G. senegalensis* offers a promising and sustainable healthcare solution.

Materials and Methods

Chemicals and reagents

All chemicals used were of analytical grade, including Folin-Ciocalteu reagent, gallic acid, aluminium chloride, methanol, and dimethyl sulfoxide (DMSO). Reagents for FTIR, GC-MS, and NMR spectroscopy were sourced from Sigma-Aldrich (St. Lous Mo, USA).

Collection, identification, and preparation of plant material

Fresh leaves of *G. senegalensis* were collected in the morning hours in September 2024 from the wild in Maiduguri, Nigeria and authenticated by a taxonomist Dr. C.A. Ukwubile of the Department of Pharmacognosy, University of Maiduguri, Nigeria. A voucher specimen number UMM/FPH/COB/001 was deposited for the plant at the herbarium of

the department. The leaves were washed, shade-dried, and pulverized into a fine powder using an electronic blender. The powder weighing 1200 g was extracted by cold maceration technique using absolute methanol to obtain a dark-green extract (102 g; yield = 8.5%).

Phytochemical screening

Qualitative phytochemical screening was conducted to detect alkaloids, flavonoids, tannins, saponins and other metabolites using standard protocols (Baeshen et al., 2023).

Determination of total phenolic and flavonoid contents

The total phenolic content (TPC) and total flavonoid content (TFC) of the *G. senegalensis* leaf extract were determined using standardized spectrophotometric methods to quantify the phenolic and flavonoid compounds, which contribute significantly to the plant's bioactivity (Zhao et al., 2022).

Total phenolic content (TPC)

The TPC was measured using the Folin-Ciocalteu method (Pillai et al., 2022). In this procedure, a stock solution of the plant extract was prepared in methanol at a concentration of 1 mg/mL. A 200 µL aliquot of the extract solution was mixed with 1 mL of Folin-Ciocalteu reagent (diluted 10-fold with distilled water). After 5 minutes, 800 µL of 7.5% sodium carbonate solution was added to neutralize the reaction mixture. The mixture was incubated in the dark at room temperature for 30 min to allow for colour development. The absorbance of the solution was then measured at 765 nm. A gallic acid standard curve (0-100 µg/mL) was prepared under the same conditions, and the TPC of the extract was calculated and expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g).

Total flavonoid content (TFC)

The TFC was determined using an aluminium chloride ($AlCl_3$) colourimetric assay, which is based on the formation of a flavonoid-$AlCl_3$ complex that produces a yellow colouration measurable at 415 nm. Briefly, a stock solution of the plant extract was prepared at 1 mg/mL in methanol. A 500 µL aliquot of the extract solution was mixed with 100 µL of 10% aluminium chloride, 100 µL of 1 M potassium acetate, and 2.8 mL of distilled water in a test tube. The mixture was incubated at room temperature for 30 minutes to facilitate the formation of the flavonoid-$AlCl_3$ complex. The absorbance of the resulting solution was measured at 415 nm using a UV-Vis spectrophotometer. A quercetin standard curve (0–100 µg/mL) was prepared, and the TFC of the extract was calculated and expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g). Both assays were performed in triplicate to ensure accuracy and reproducibility (Pillai et al., 2022).

Isolation and purification of polyphenols

The isolation and purification of polyphenols from the *G. senegalensis* leaf extract involved a series of steps designed to concentrate and purify these bioactive compounds while minimizing interference from non-phenolic components. The procedure combined solvent extraction, liquid-liquid partitioning, and chromatographic techniques to ensure a high degree of purity and yield (Abuzaid et al., 2020).

Extraction of polyphenols

The initial extraction of polyphenols was carried out using methanol, which is a polar solvent effective in extracting a wide range of phenolic compounds. Dried and powdered leaves of *G. senegalensis* were macerated with 80% methanol (v/v) in a 1:10 ratio (w/v) at room temperature for 48 h with occasional stirring. The mixture was filtered through Whatman No. 1 filter paper to remove plant debris, and the filtrate was collected. The extraction process was repeated twice to maximize polyphenol recovery, and the combined filtrates were concentrated under reduced pressure using a rotary evaporator at 40 °C to obtain a crude methanolic extract (State et al., 2023).

Liquid-liquid partitioning (LLP)

To fractionate the crude methanolic extract and enrich the polyphenol content, liquid-liquid partitioning was performed. The concentrated extract was dissolved in distilled water and sequentially partitioned with solvents of increasing polarity: hexane, ethyl acetate, and n-butanol. Each fraction was concentrated separately under reduced pressure, and the

ethyl acetate fraction, which exhibited the highest phenolic content in preliminary screening, was selected for further purification (Keskes et al., 2017).

Purification by silica gel column chromatography

Polyphenols in the ethyl acetate fraction were further purified using column chromatography on silica gel. Silica gel (60-120 mesh size) was used as the stationary phase, and the column was packed using the slurry method with hexane as the packing solvent. The ethyl acetate fraction was dissolved in a minimal amount of ethyl acetate and loaded onto the silica gel column. Elution was carried out using a gradient of solvents, starting with hexane, and gradually increasing the polarity with mixtures of hexane: ethyl acetate (95:5 to 0:100), followed by ethyl acetate: methanol (95:5 to 0:100). Fractions (20 mL) were collected at regular intervals and monitored using thin-layer chromatography (TLC) with a phenolic detection reagent, such as ferric chloride. Fractions showing similar TLC profiles were pooled, concentrated under reduced pressure, and subjected to preliminary spectroscopic analysis (UV-Vis and FTIR) to confirm the presence of polyphenols (Amedu et al., 2024; Keskes et al., 2017; Rasul, 2018).

Final purification of fractions

The pooled fractions containing enriched polyphenols were further purified using preparative high-performance liquid chromatography (HPLC) to achieve higher purity. The purified polyphenols were dried, weighed, and stored at 4 °C in amber vials for subsequent characterization and biological assays. This multi-step approach ensured the isolation of high-quality polyphenols, suitable for evaluating their cytotoxic and anti-inflammatory activities (Brito et al., 2018).

Characterization of polyphenols

The purified polyphenols were characterized to determine their structural and functional properties using a combination of advanced analytical techniques.

Fourier-transform infrared (FTIR) spectroscopy

This was employed to identify functional groups using an Alpha II FTIR (Bruker, USA) by analyzing characteristic absorption bands from 4000-500 wavenumber cm^{-1} corresponding to hydroxyl, aromatic, and other functional moieties typical of polyphenols (Krishnan et al., 2016).

GC-MS analysis

The chemical composition of the sample was analyzed using an Agilent Technologies 7890A GC-MS system equipped with an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm film thickness). The sample (compound from extract) was prepared by dissolving approximately 2 mg in 1 mL of high-purity methanol (Sigma Aldrich St Lous Mo, USA), followed by filtration through a 0.22 µm PTFE syringe filter. A 1 µL aliquot of the prepared solution was injected into the GC-MS system in split mode with a split ratio of 10:1. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The oven temperature was initially set at 60 °C and held for 2 minutes before being increased at a rate of 10°C/min to a final temperature of 300 °C, which was maintained for 5 min. Mass spectrometric detection was carried out using electron impact ionization (70 eV), scanning a mass range of 50–5000 m/z. The chromatographic data and mass spectra were analyzed using Agilent Mass Hunter software, and compounds were identified by comparing the observed spectra to those in the NIST library (Ukwubile et al., 2019).

NMR analysis

The molecular structure of the sample was confirmed using a Bruker 850 MHz NMR spectrometer equipped with an advanced cryoprobe. The sample was prepared by dissolving approximately 10 mg in 0.5 mL of deuterated DMSO (DMSO-d6) and transferring the solution into a 5 mm NMR tube. The system was calibrated using tetramethyl silane (TMS) as an internal reference. High-resolution ^{1}H NMR spectra were recorded with a spectral width of 0–12 ppm, using 32 scans and a relaxation delay of 2 sec. Similarly, ^{13}C NMR spectra were acquired within a spectral width of 0–200 ppm, using 512 scans to enhance the signal-to-noise ratio. The resulting spectra were processed and analyzed using Bruker TopSpin software. The chemical shifts, coupling constants, and integration values provided detailed insights into the hydrogen and carbon atom arrangements within the polyphenolic framework. The combined data confirmed the expected structural features and molecular connectivity (Ukwubile et al., 2024).

In vitro cytotoxicity by MTT assay

The cytotoxicity of polyphenols was evaluated using the MTT assay on MCF-7 (breast cancer) and OV7 (ovarian cancer) cell lines. Cells were seeded in 96-well plates at a density of 1×10^4 cells/well and incubated overnight at 37 °C in a humidified atmosphere with 5% CO_2 to allow adherence. Polyphenols were prepared in a range of concentrations (10–200 µg/mL) and added to the wells, followed by a 48-hour incubation. After treatment, 20 µL of MTT solution (5 mg/mL in PBS) was added to each well and incubated for 4 hours. The medium was carefully removed, and the formazan crystals formed by viable cells were dissolved in 100 µL of dimethyl sulfoxide (DMSO). Absorbance was measured at 570 nm using a microplate reader. The percentage of cell viability was calculated relative to untreated control cells. IC_{50} values were determined from dose-response curves (Nelson et al., 2020).

Transwell migration assay

The Transwell migration assay was performed to assess the anti-metastatic potential of the polyphenols. Transwell chambers with an 8 µm pore size were used. Cancer cells (1×10^5) suspended in serum-free medium were placed in the upper chamber, while the lower chamber was filled with medium containing 10% fetal bovine serum (FBS) as a chemoattractant. Polyphenols at non-cytotoxic concentrations were added to the upper chamber. After 24 hours of incubation, non-migrated cells on the upper side of the membrane were removed using a cotton swab. Migrated cells on the lower side were fixed with 4% paraformaldehyde, stained with crystal violet, and counted under a microscope in five random fields (Justus et al., 2014).

Caspase-3 activity evaluation

Caspase-3 activity was measured using a colourimetric assay kit (ThermoFisher Scientific, USA). Treated and untreated cells were lysed, and the lysates were incubated with a caspase-3-specific substrate (Ac-DEVD-pNA; Sigma, USA). The cleavage of the substrate by active caspase-3 released a chromophore (pNA), which was measured spectrophotometrically at 405 nm. Increased caspase-3 activity in treated cells indicated apoptosis induction (Jiang et al., 2017).

Apoptosis detection by flow cytometry

Apoptosis was detected using Annexin V-FITC/PI dual staining. Cells treated with polyphenols were harvested, washed with PBS, and resuspended in a binding buffer. Annexin V-FITC and propidium iodide (PI) were added to the cell suspension and incubated in the dark for 15 min at 40 °C. The stained cells were analyzed by flow cytometry, with Annexin V-FITC indicating early apoptosis and PI identifying late apoptosis or necrosis (Kotowski et al., 2017).

Other in vitro assays

Reactive oxygen species (ROS) generation: Intracellular ROS levels were measured using 2',7'-dichlorofluorescin diacetate (DCFDA). Treated cells were incubated with DCFDA, and fluorescence intensity was measured using a fluorometer, indicating oxidative stress *(*Baothman et al., 2023).

Cell cycle analysis: Flow cytometry was used to analyze cell cycle arrest. Treated cells were fixed in ethanol, and stained with PI, and DNA content was measured to determine cell cycle distribution (Jiang et al., 2017).

Colony formation assay: Cancer cells treated with polyphenols were seeded in 6-well plates and allowed to form colonies over 10–14 days. Colonies were fixed, stained, and counted to evaluate long-term proliferation inhibition (Wang et al., 2024).

Experimental animals

Adult Wistar rats (weighing 150–200 g) were used for the acute and subacute toxicity studies. The animals were housed in standard polypropylene cages under controlled environmental conditions (temperature 25 ± 2°C, relative humidity 55 ± 5%, and a 12-hour light/dark cycle) with free access to a standard pellet diet and water *ad lib*. Before the experiments, the animals were acclimatized for one week. All experimental procedures were conducted in compliance with institutional guidelines and approved by the Institutional Animal Ethics Committee (IAEC) following the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals.

Acute oral and subacute toxicity studies of G. senegalensis extract

The acute and subacute toxicity studies of *Guiera senegalensis* extract were conducted in Wistar rats following the Organization for Economic Cooperation and Development (OECD) guidelines. For the acute toxicity study, rats were divided into groups (n=5 per group) and administered a single oral dose of the extract at varying doses (200, 500, 1000, and 2000 mg/kg body weight). The animals were observed for 24 hours for signs of toxicity, behavioural changes, and mortality, followed by monitoring for 14 days. For the subacute toxicity study, rats were randomly assigned into groups (n = 6 per group) and administered the extract orally at doses of 250, 500, and 1000 mg/kg body weight daily for 28 consecutive days. Body weights were recorded weekly, and the animals were observed for clinical signs of toxicity throughout the study. At the end of the study, blood samples were collected via cardiac puncture under anaesthesia for haematological and biochemical analyses. Vital organs (liver, kidney, heart, lungs, and spleen) were excised, weighed, and examined for histopathological changes.

In vivo anti-inflammatory assays

Anti-inflammatory activities were assessed using carrageenan-induced paw oedema, NO inhibition, and formalin-induced paw-licking tests in Wistar rats (Yimer et al., 2020).

Carrageenan-induced paw oedema

The anti-inflammatory activity of the polyphenols was evaluated using the carrageenan-induced paw oedema model in Wistar rats. Adult rats (180–200 g) were divided into five groups (n = 5 per group): normal control, carrageenan control, standard drug (indomethacin at 10 mg/kg), polyphenol-treated low dose, and polyphenol-treated high dose groups. Before the experiment, the animals were fasted overnight with water *ad libitum*. Paw oedema was induced by subcutaneous injection of 0.1 mL of 1% carrageenan solution into the plantar region of the right hind paw. The test groups received oral doses of polyphenols (50 and 100 mg/kg b.w.) 1 h before the carrageenan injection, while the control group received only the vehicle. Paw thickness was measured using a digital vernier caliper at 0, 1, 2, 3, and 4 h after the carrageenan injection. The percentage inhibition of paw oedema was calculated using the formula below:

$$\%\ inhibition\ = \frac{mean\ paw\ volume\ (control)\ -\ mean\ paw\ volume\ (treatment)}{mean\ paw\ volume\ (control)}\ x\ 100$$

Nitric oxide (NO) inhibition assay

Nitric oxide (NO) levels were measured in the plasma of rats as an indicator of anti-inflammatory activity. After carrageenan injection, blood samples were collected via retro-orbital puncture at the 4-hour time point and centrifuged at 3000 rpm for 15 min to obtain plasma. The NO concentration in plasma was determined using the Griess reaction. Briefly, equal volumes of plasma and Griess reagent (1% sulfanilamide and 0.1% N-(1-naphthyl) ethylenediamine in 2.5% phosphoric acid) were mixed and incubated at room temperature for 10 min. The absorbance of the resulting pink chromophore was measured at 540 nm using a UV-vis spectrophotometer. A standard curve prepared with sodium nitrite was used to calculate NO levels. The concentration of NO was calculated from the formula below (Bourgou et al., 2012):

$$NO\ levels\ (\mu M)\ = \frac{absorbance\ of\ sample\ -\ absorbance\ of\ blank}{slope\ of\ standard\ curve}\ x\ dilutionfactor$$

Formalin-induced paw-licking test

The analgesic and anti-inflammatory effects of polyphenols were further assessed using the formalin-induced paw-licking model. Wistar rats were divided into similar groups as described above. The test compound and control substances were administered 1 h before formalin injection. To induce pain and inflammation, 20 µL of 2.5% formalin was injected subcutaneously into the plantar surface of the right hind paw. The animals were observed for two distinct phases of paw-licking behaviour: the early phase (0–5 min), representing neurogenic pain and the late phase (15–30 min), reflecting inflammatory pain. The duration of paw licking in each phase was recorded. A significant reduction in paw licking time in the treated groups compared to the control group was considered indicative of anti-inflammatory and analgesic effects (Borquaye et al., 2020).

Evaluation of neurotransmitter and inflammatory markers

These markers: TH (tyrosine hydroxylase), TPH (tryptophan hydroxylase), DAT (dopamine transporter), SERT (serotonin transporter), Iba1 (ionized calcium-binding adaptor molecule 1), GFAP (glial fibrillary acidic protein), iNOS (inducible nitric oxide synthase), Caspase-3, BDNF (brain-derived neurotrophic factor), and NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells). The evaluation of these markers was performed using three methods: ELISA (enzyme-linked immunosorbent assay), immunohistochemistry, and western blotting as outlined below (Jayachandran et al., 2023):

ELISA assay

ELISA was used to quantify markers such as BDNF, iNOS, and NF-κB in tissue homogenates or serum samples. Brain tissues were homogenized in a lysis buffer containing protease and phosphatase inhibitors and centrifuged to collect the supernatant, which was used for analysis. Protein concentrations were determined using a Bradford assay before the ELISA procedure. In this method, 96-well plates were coated with capture antibodies specific to the target marker and incubated overnight at 4 °C. After washing, blocking buffer was added to reduce non-specific binding. Supernatants and standards were added to the wells and incubated, followed by detection antibodies conjugated to an enzyme such as HRP. A substrate like TMB was then added to produce a colourimetric reaction. The reaction was stopped using an acid (1N HCl), and absorbance was measured at 450 nm. Marker concentrations were calculated using a standard curve prepared with known concentrations of the target protein.

Immunohistochemistry (IHC)

IHC was performed to localize and visualize markers such as TH, GFAP, and Iba1 in brain tissue sections. Tissues were fixed in 10% formalin, embedded in paraffin, and sectioned into 4-μm-thick slices. These sections were deparaffinized in xylene and rehydrated in graded ethanol. Antigen retrieval was carried out using citrate buffer (pH 6.0) in a microwave or pressure cooker to enhance antibody binding. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide. The sections were then incubated overnight at 4 °C with primary antibodies specific to the target marker. Biotinylated secondary antibodies and streptavidin-HRP were added, followed by a chromogenic substrate such as DAB to visualize antigen-antibody interactions. Tissues were counterstained with hematoxylin, dehydrated, and mounted for microscopic analysis. Marker expression was quantified by evaluating the percentage of positively stained cells or optical density using image analysis software (Hassanen et al., 2024).

Western blot analysis

Western blotting was used to evaluate the protein expression levels of markers like TPH, DAT, SERT and caspase-3 Brain tissues were homogenized in RIPA buffer containing protease and phosphatase inhibitors. The lysates were centrifuged, and the supernatant containing soluble proteins was collected. Protein concentrations were determined using a Bradford or BCA assay. Equal amounts of protein (30–50 μg per sample) were separated on 10–12% SDS-PAGE gels and transferred onto PVDF or nitrocellulose membranes. Membranes were blocked in 5% non-fat milk or BSA to reduce non-specific binding and incubated overnight at 4 °C with primary antibodies specific to the target markers. After washing, membranes were incubated with HRP-conjugated secondary antibodies, and the signals were detected using an enhanced chemiluminescence (ECL) system. Band intensities were visualized using a gel documentation system and analyzed using densitometry software such as ImageJ. Protein expression levels were normalized to housekeeping proteins such as β-actin or GAPDH for comparison across groups (Lim et al., 2019).

Statistical analysis

Data obtained were expressed as means ± SD (n = 3). The values of $p < 0.05$ or $p < 0.01$ were taken as statistically significant when compared to control using one-way ANOVA followed by Dunnett's post hoc test.

Results

Phytochemical content

The phytochemical screening of the *G. senegalensis* leaf extract partitioned into n-hexane (HF), ethyl acetate (EF), and n-butanol (BF) fractions revealed a diverse distribution of metabolites (Table 1). Alkaloids, flavonoids, tannins, and phenols were present in the EF and BF fractions, highlighting their affinity for moderately and highly polar solvents.

Saponins, glycosides, and carbohydrates were exclusively found in the polar BF fraction, indicating their solubility in water-miscible solvents. Nonpolar compounds such as terpenoids and steroids were predominantly extracted into the HF and EF fractions. Anthraquinones were detected only in the EF fraction, suggesting a preference for moderately polar solvents. The absence of certain metabolites, such as alkaloids and flavonoids, in the HF fraction further underscores the selective extraction capabilities of the solvents used. This partitioning approach effectively separates and identifies a wide range of secondary metabolites based on their polarity.

Table 1. Phytochemical content of *G. senegalensis* methanol leaf extract partitioned in solvents of varying polarities

Constituent	Test	HF	EF	BF
Alkaloids	Wagner's	-	+	+
Flavonoids	Shinoda	-	+	+
Tannins	Ferric chloride	-	+	+
Saponins	Frothing	-	-	+
Terpenoids	Salkowski	+	+	-
Steroids	Liebermann-Burchard	+	+	-
Phenols	Ferric chloride	-	+	+
Glycosides	Keller-Killiani	-	-	+
Anthraquinones	Bontrager	-	+	-
Carbohydrates	Molisch's	-	-	+

+ (present), **-** (absent), HF (hexane fraction), EF (ethyl acetate fraction) and BF (n-butanol fraction).

Total phenolic flavonoid contents

The total phenolic content (TPC) and total flavonoid content (TFC) of *G. senegalensis* leaf methanol extract was quantified within the concentration range of 0–100 µg/mL, demonstrating a dose-dependent increase in phenolic and flavonoid levels (Table 2). At the highest tested concentration (100 µg/mL), the extract exhibited a TPC value of 352.32 ± 2.41 mg GAE/g and a TFC value of 56.74 ± 1.01 mg QE/g when compared to other fractions indicating that *G. senegalensis* is a rich source of phenolic and flavonoid compounds. The hexane fraction did not contain detectable levels of phenolic or flavonoid compounds, as these compounds are polar and were retained in more hydrophilic fractions. The ethyl acetate fraction had the highest enrichment of phenolic and flavonoid content, indicating its effectiveness in extracting intermediate polarity compounds. The purified polyphenols exhibited significantly enhanced phenolic and flavonoid content, underscoring the efficacy of the liquid-liquid partitioning and chromatographic purification methods.

Table 2. Number of polyphenols from liquid-liquid partitioning of *G. senegalensis* leaf methanol extract at 0-100 µg/mL

Fraction	Weight (g)	Yield (%)	TPC (mg GAE/g)	TFC (mg QE/g)
ME	50.0	100	352.32 ± 2.41	56.74 ± 1.01
HF	5.2	10.4	0.00 ± 0.00	0.00 ± 0.00
EF	15.8	31.6	78.3 ± 2.03	56.4 ± 1.02
BF	10.5	21.0	45.2 ± 1.03	32.5 ± 1.01
AF	18.5	37.0	15.6 ± 1.01	10.8 ± 0.01
Purified polyphenols	2.5	5.0	145.7 ± 3.11	102.4 ± 2.01

ME (methanol extract), HF (hexane fraction), EF (ethyl acetate fraction) and BF (n-butanol fraction)

Isolation and characterization of polyphenols

The ethyl acetate fraction (EF) of *G. senegalensis* was subjected to comprehensive structural characterization to identify its polyphenolic constituents. After purification through column chromatography and preparative HPLC, individual polyphenols were analyzed using FTIR, GC-MS, and NMR spectroscopy as shown below:

FTIR analysis

The FTIR spectrum of the ethyl acetate fraction (Fig. 1) revealed key functional groups characteristic of polyphenols. A broad peak at 3400–3300 wavenumber cm^{-1} was attributed to hydroxyl (-OH) stretching, indicative of phenolic compounds. Peaks at 2920 cm^{-1} and 2850 wavenumber cm^{-1} corresponded to C-H stretching vibrations in aliphatic

chains. Distinct absorption bands at 1620–1600 wavenumber cm^{-1} were associated with aromatic C=C stretching, confirming the presence of aromatic rings. Additionally, sharp bands at 1270–1210 wavenumber cm^{-1} and 1120–1080 wavenumber cm^{-1} were assigned to C-O stretching in phenolic ethers and esters. The functional group assignments strongly suggested the presence of flavonoids and phenolic acids.

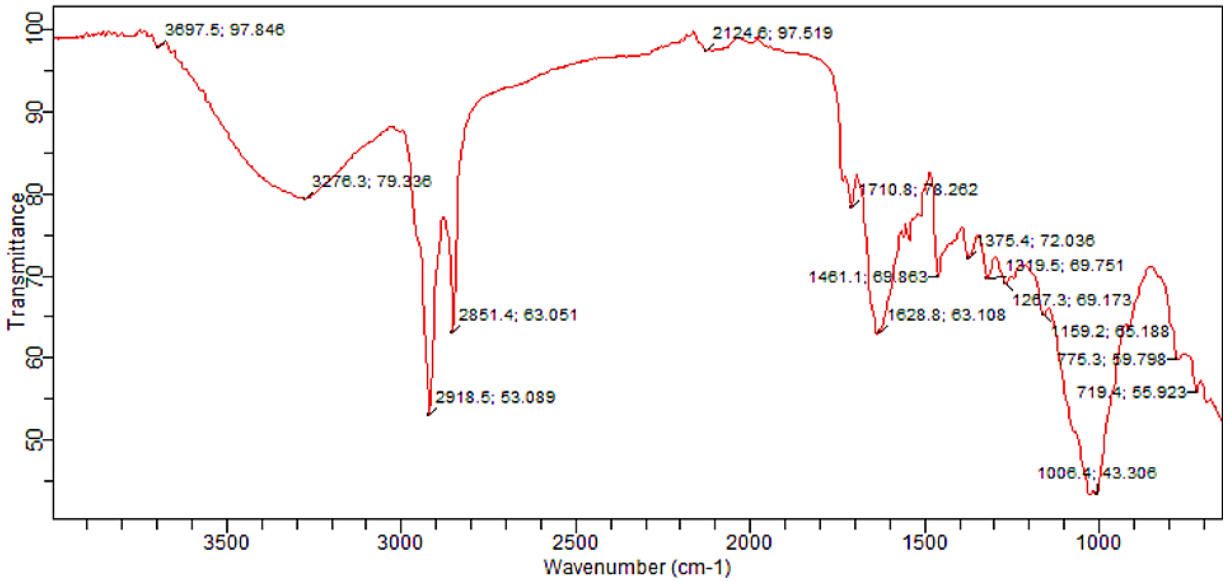


Figure 1. FTIR spectrum of isolated polyphenols from *G. senegalensis* leaf extract

GC-MS profiling

Gas chromatography-mass spectrometry (GC-MS) identified several bioactive compounds within the ethyl acetate fraction. Key polyphenolic constituents included gallic acid, quercetin, catechin, and kaempferol derivatives (Fig. 2). These compounds were identified based on their mass fragmentation patterns and retention times, which were matched against established compound libraries. Gallic acid (RT: 18.33 min, m/z 170.01), quercetin (RT: 22.51 min, m/z 302.08), catechin (RT: 24.12 min, m/z 290.28) and Kaempferol (RT: 24.70 min, m/z 286.12).

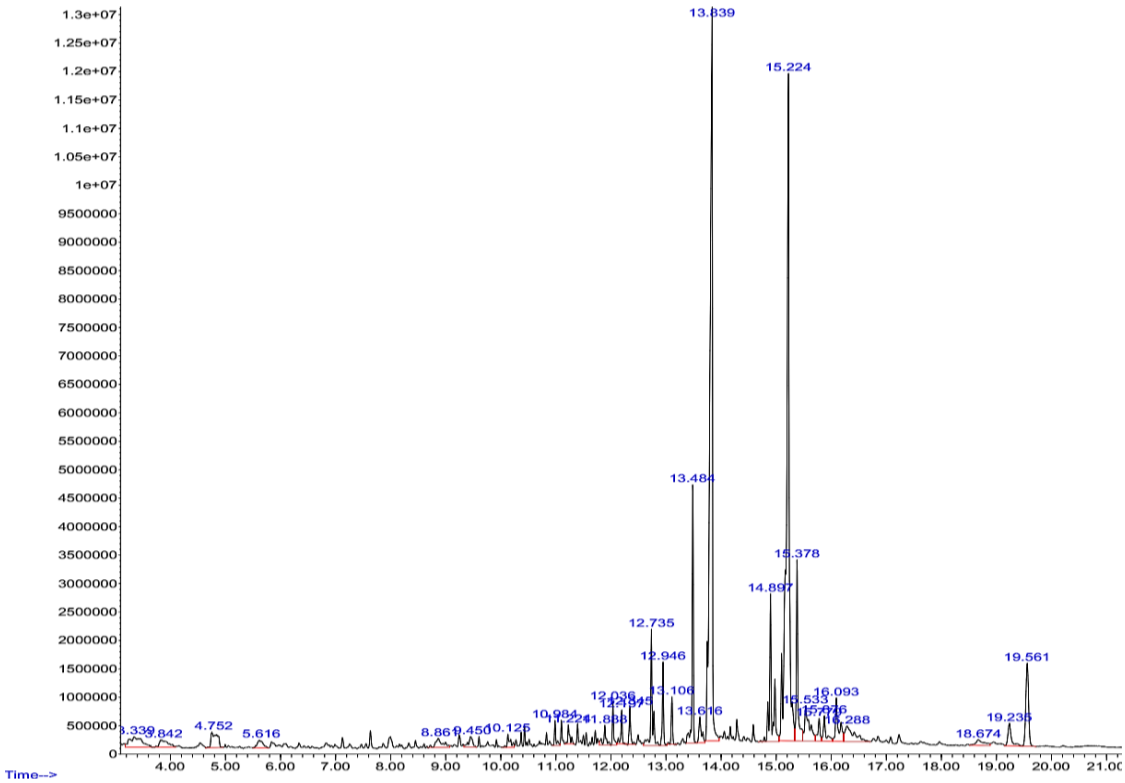


Figure 2. GC-MS chromatogram of isolated polyphenols from *G. senegalensis* leaf extract

NMR spectroscopy

The ^{1}H and ^{13}C NMR spectra (Fig. 3 a and b) provided detailed structural information about the isolated polyphenols. The ^{1}H NMR spectrum exhibited key signals: A singlet at **δ** 7.30 ppm, indicative of hydroxyl protons on the aromatic ring (as in gallic acid), multiplets at **δ** 6.50–7.50 ppm, corresponding to aromatic protons in quercetin and kaempferol, doublets at **δ** 5.90–6.10 ppm, characteristic of catechin's aromatic hydrogens and signals at **δ** 3.70–4.20 ppm, suggesting protons in aliphatic hydroxyl groups. Similarly, the ^{13}C NMR spectrum (Fig. 3b) confirmed the presence of phenolic carbons, with signals at **δ** 145–160 ppm attributed to hydroxylated aromatic carbons and **δ** 110–135 ppm assigned to non-hydroxylated aromatic carbons. Peaks at **δ** 170–180 ppm corresponded to carboxyl (-COOH) carbons, consistent with phenolic acids like gallic acid. Overall, the FTIR, GC-MS, and NMR analyses conclusively identified major polyphenols such as gallic acid, quercetin, catechin, and kaempferol in the ethyl acetate fraction of *G. senegalensis*. These findings highlight the presence of diverse bioactive compounds.

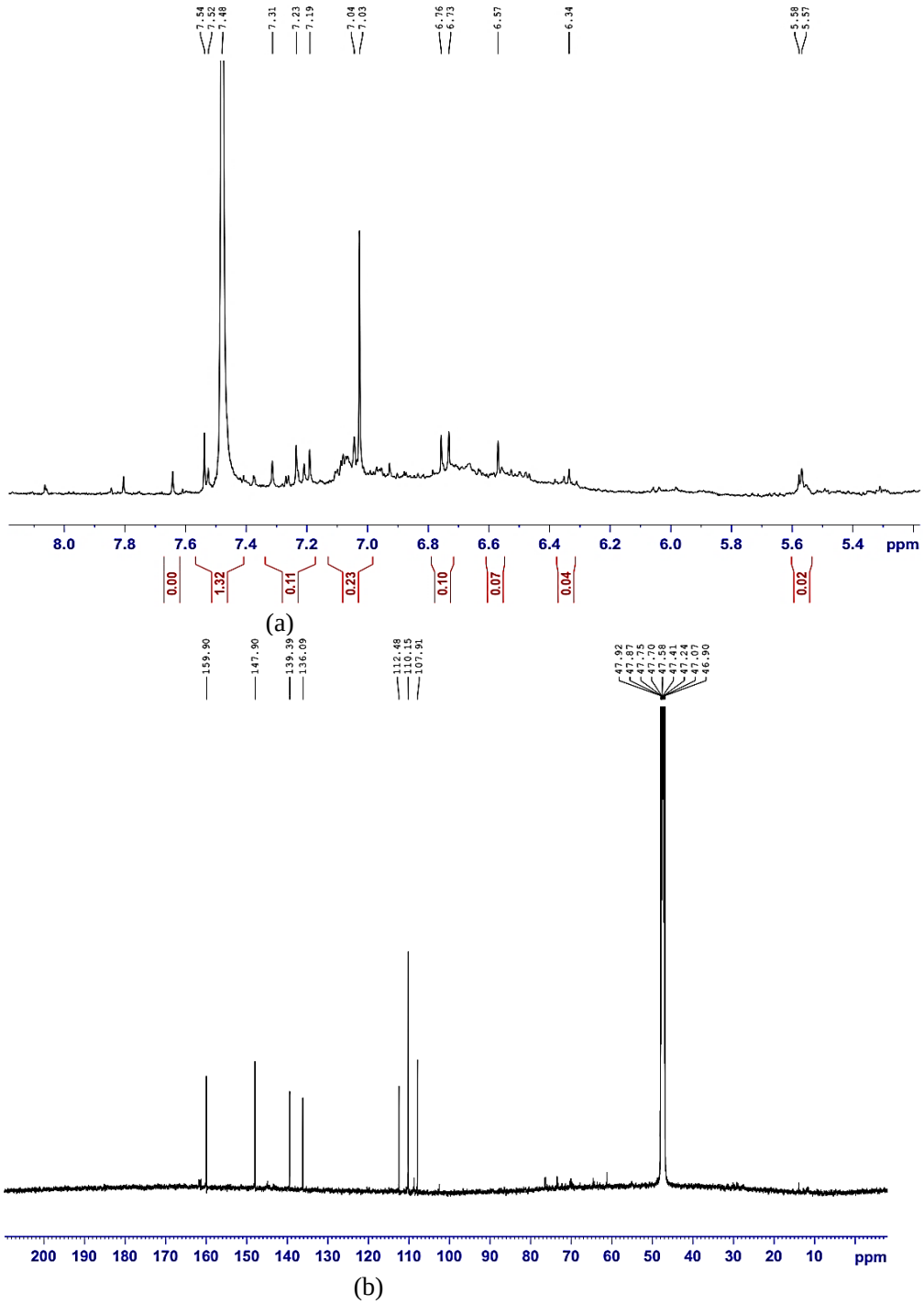


Figure 3. (a) ^{1}H and (b) ^{13}C-NMR spectra of an isolated polyphenol from *G. senegalensis* leaf extract

Acute oral and subacute toxicities

The acute toxicity study showed no mortality at lower doses (200–1000 mg/kg), but signs of lethargy and tremors were observed at 2000 mg/kg, with a 20% mortality rate. Subacute toxicity revealed dose-dependent changes in haematological parameters, body weight, and organ weights, particularly at 1000 mg/kg (Tables 3-5; Fig. 4). Histopathological analysis confirmed tissue alterations in the liver, kidney, and spleen at higher doses, suggesting possible dose-dependent toxicity (Fig. 5).

Table 3. Acute toxicity study of *G. senegalensis* leaf extract (24-hour and 14-day observations)

Dose (mg/kg)	Mortality (%)	Behavioural changes observed	Food/water intake	Body weight change (%)
Normal control	0	None	Normal	0
200	0	None	Normal	+2.1
500	0	Slight sedation (1–2 h)	Reduced (initial)	+1.8
1000	0	Sedation, reduced mobility	Reduced (initial)	-1.2
2000	20	Lethargy, tremors	Reduced	-5.0

Table 4. Effect of *G. senegalensis* leaf extract on rats' haematological parameters after 28 days

Parameter	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Hemoglobin (g/dL)	13.5 ± 0.3	13.3 ± 0.4	12.8 ± 0.5	12.1 ± 0.6*
RBC (10^6/µL)	6.5 ± 0.2	6.4 ± 0.2	6.1 ± 0.3	5.7 ± 0.3*
WBC (10^3/µL)	8.5 ± 0.5	8.3 ± 0.4	8.0 ± 0.6	7.2 ± 0.7*
Platelets (10^5/µL)	3.2 ± 0.1	3.1 ± 0.1	2.9 ± 0.2	2.7 ± 0.2*

Values are expressed as mean ± SD. * indicates significant differences compared to the control group using one-way ANOVA followed by Dunnett's post hoc test ($p<0.05$)

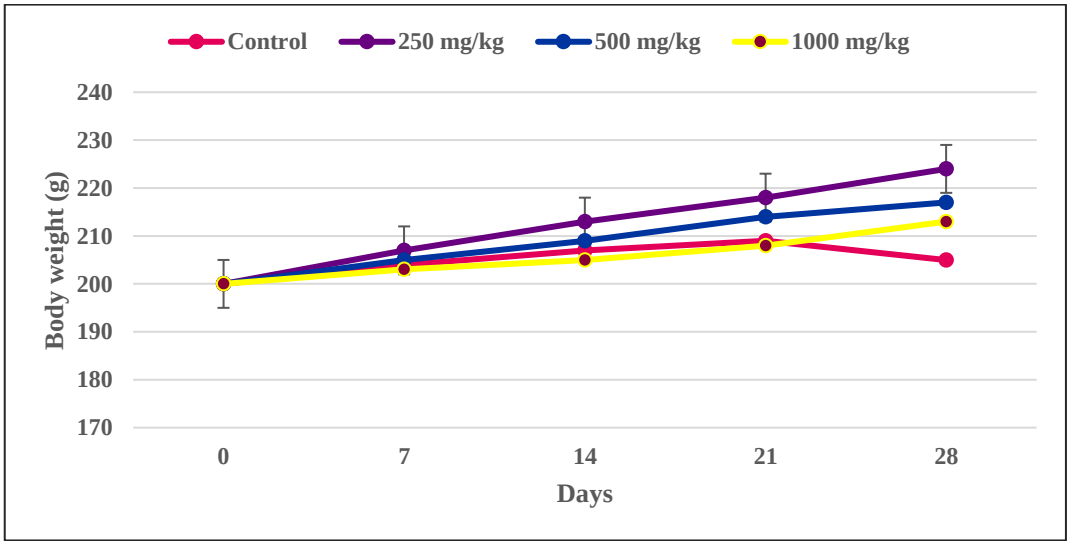


Figure 4. Changes in body weight during subacute toxicity study of *G. senegalensis* leaf extract on Wistar rats. Results are means ± SD (n = 6)

Table 5. Effect of *G. senegalensis* leaf extract on organ weight after 28 days subacute study

Organ	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Liver	3.5 ± 0.2	3.4 ± 0.1	3.3 ± 0.2	3.1 ± 0.2*
Kidney	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.1*
Heart	0.4 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.0*
Lungs	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1*
Spleen	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.0	0.4 ± 0.0*

Values are expressed as mean ± SD. * indicates significant differences compared to the control group using one-way ANOVA followed by Dunnett's post hoc test ($p<0.05$)

In Figure 5 below, the liver showed normal architecture with no hepatocyte degeneration or necrosis. The kidney showed mild glomerular atrophy, tubular damage, and inflammation while the heart and spleen maintained red pulp integrity in both control and treated groups.

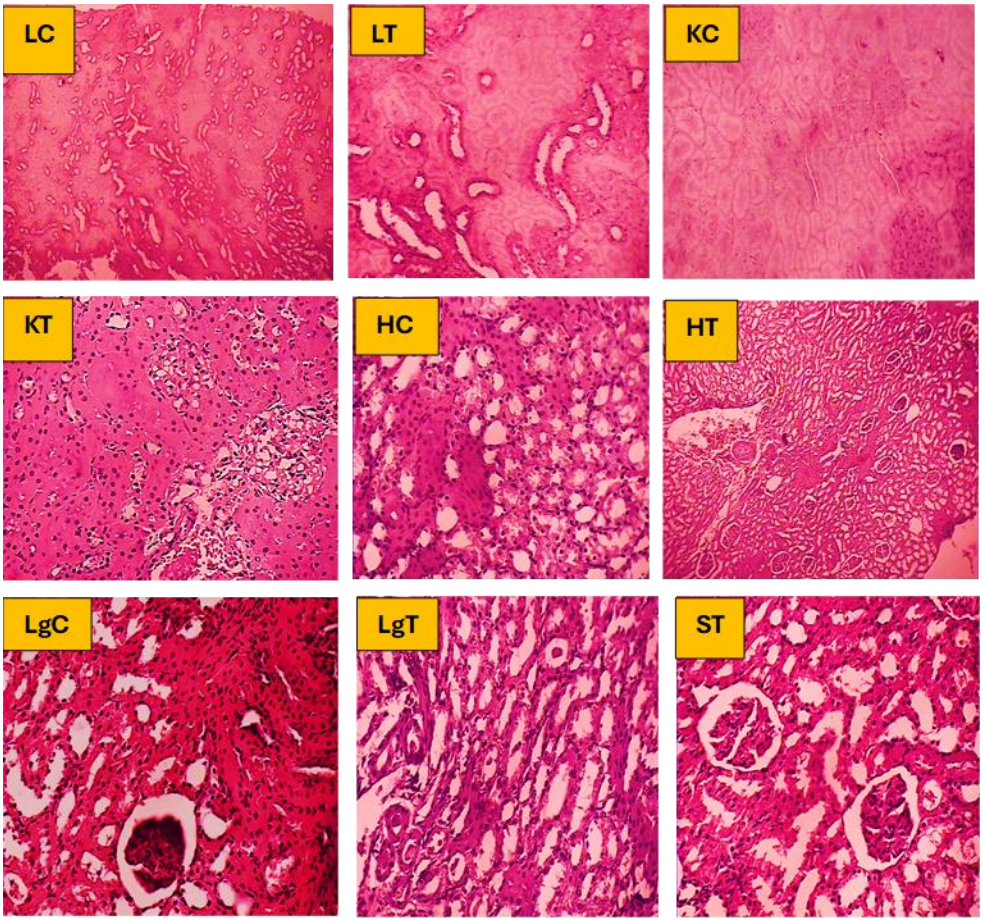


Figure 5. Photomicrographs of vital Organs at high dose of 100 mg/kg b.w. *G. senegalensis* leaf extract after 28 days

LC: liver control, LT: liver treated, KC: kidney control, KT: kidney treated, HC: heart control, HT: heart treated, LgC: lung control, LgT: lung treated, and ST: spleen treated, 400x.

Cytotoxic effects

Cell viability by MTT assay

The MTT assay demonstrated the dose-dependent cytotoxic effects of *G. senegalensis* polyphenols on MCF-7 and OV7 cancer cell lines, compared to the standard drug, doxorubicin. For MCF-7 cells, minimal cytotoxicity was observed at 10 µg/mL, with cell viability at 90.5%. A gradual decline was observed at 25 µg/mL (78.4%) and 50 µg/mL (62.7%). Significant cytotoxic effects were noted at 100 µg/mL (48.3%), which closely approximated the IC_{50} value. At 200 µg/mL, viability dropped drastically to 29.5%, indicating potent cytotoxicity. Doxorubicin at its IC_{50} concentration exhibited a viability of 45%, demonstrating comparable efficacy. For OV7 cells, cell viability was slightly higher at lower concentrations, with 92.3% at 10 µg/mL and 80.5% at 25 µg/mL. A substantial decline was observed at 50 µg/mL (64.2%) and 100 µg/mL (50.7%), with the IC_{50} approximated at this concentration. At 200 µg/mL, cell viability decreased significantly to 32.1%, showing strong cytotoxic effects. Doxorubicin at its IC_{50} concentration resulted in a cell viability of 46% for OV7 cells, suggesting similar efficacy to the polyphenols.

Table 6. Cell viability of MCF-7 and OV7 cancer cell lines treated with G. senegalensis polyphenols and doxorubicin by MTT assay

Concentration (µg/mL)	MCF-7 viability - polyphenols (%)	MCF-7 viability - doxorubicin (%)	OV7 viability - polyphenols (%)	OV7 viability - doxorubicin (%)
10	90.5 ± 2.3	88.2 ± 2.5	92.3 ± 2.4	89.5 ± 2.6
25	78.4 ± 3.1	74.8 ± 3.0	80.5 ± 3.0	76.3 ± 2.9
50	62.7 ± 2.9	58.1 ± 2.8	64.2 ± 3.1	60.7 ± 3.0
100	48.3 ± 3.0	45.0 ± 2.4	50.7 ± 2.8	47.2 ± 2.7
200	29.5 ± 2.8	27.3 ± 2.7	32.1 ± 3.0	30.2 ± 2.9

Results are means ± SD (n = 3)

Transwell migration and invasion

The transwell migration assay results (Table 7) demonstrate a significant reduction in the migration of MCF-7 and OV7 cell lines with increasing concentrations of the test treatment. At the highest concentration of 200 µg/mL, migration decreased dramatically to 50 cells for MCF-7 and 30 cells for OV7, compared to 500 and 600 cells, respectively, in the untreated control group. Doxorubicin used as a reference drug, exhibited comparatively less inhibition of migration, especially at lower concentrations. The percentage inhibition data (Table 8; Fig. 6) confirm a dose-dependent effect of the treatment on both cell lines. Maximum inhibition was observed at 200 µg/mL, with 80% inhibition in MCF-7 and 88% in OV7 cells. Notably, the test treatment showed superior migration inhibition compared to doxorubicin at equivalent concentrations. This indicates the potential efficacy of the treatment in suppressing cancer cell migration in both breast (MCF-7) and ovarian (OV7) cancer models.

Table 7. Migration of MCF-7 and OV7 cell lines in response to different treatments

Concentration (µg/mL)	MCF-7 migration	OV7 migration
0 (Control)	500	600
50	400	350
100	250	205
150	200	102
200	50	30
Doxorubicin 50	600	650
Doxorubicin 100	400	500

Table 8. Percentage inhibition of migration in MCF-7 and OV7 cell lines

Concentration (µg/mL)	MCF-7 inhibition (%)	OV7 inhibition (%)
0 (Control)	0	0
50	10	25
100	60	45
150	75	76
200	80	88
Doxorubicin 50	20	25
Doxorubicin 100	35	30

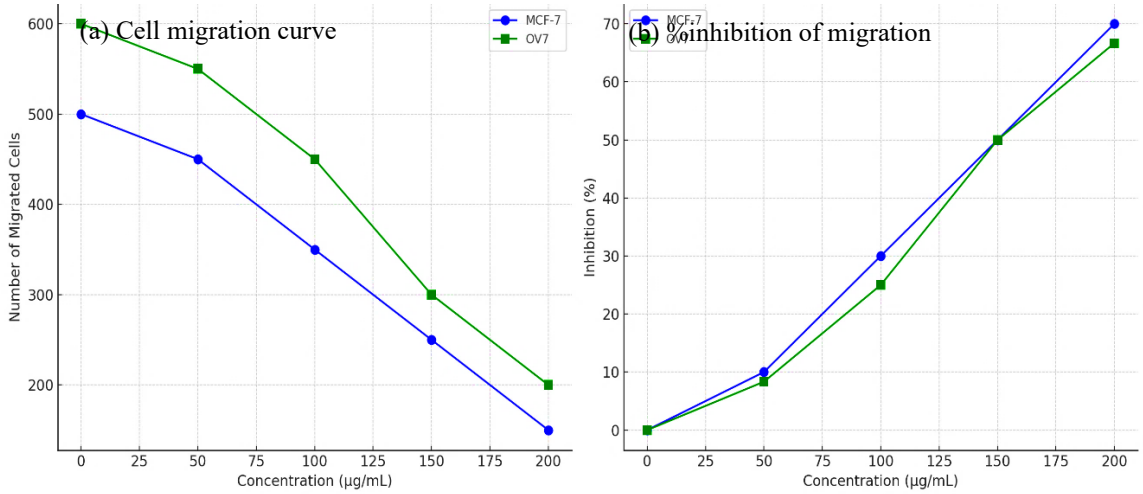


Figure 6. Effects of *G. senegalensis* polyphenol on cellular migration

Effects on caspase-3 like activity

The effects of polyphenols extracted from *Guiera senegalensis* and doxorubicin on caspase-3-like activity in MCF-7 and OV7 cell lines were evaluated across concentrations of 0–200 µg/mL (Fig. 7). Results indicate a dose-dependent increase in caspase-3 like activity for both cell lines, with the polyphenols exhibiting greater efficacy compared to doxorubicin. At the highest concentration (200 µg/mL), the polyphenols significantly ($p < 0.05$ and $p < 0.01$) enhanced caspase-3-like activity, surpassing the levels induced by doxorubicin. The increase in activity suggests that *G.*

senegalensis polyphenols effectively promote apoptosis, potentially contributing to their anticancer effects. In contrast, doxorubicin showed moderate effects, particularly at lower concentrations, highlighting the superior apoptotic potential of the polyphenols in these cancer models.

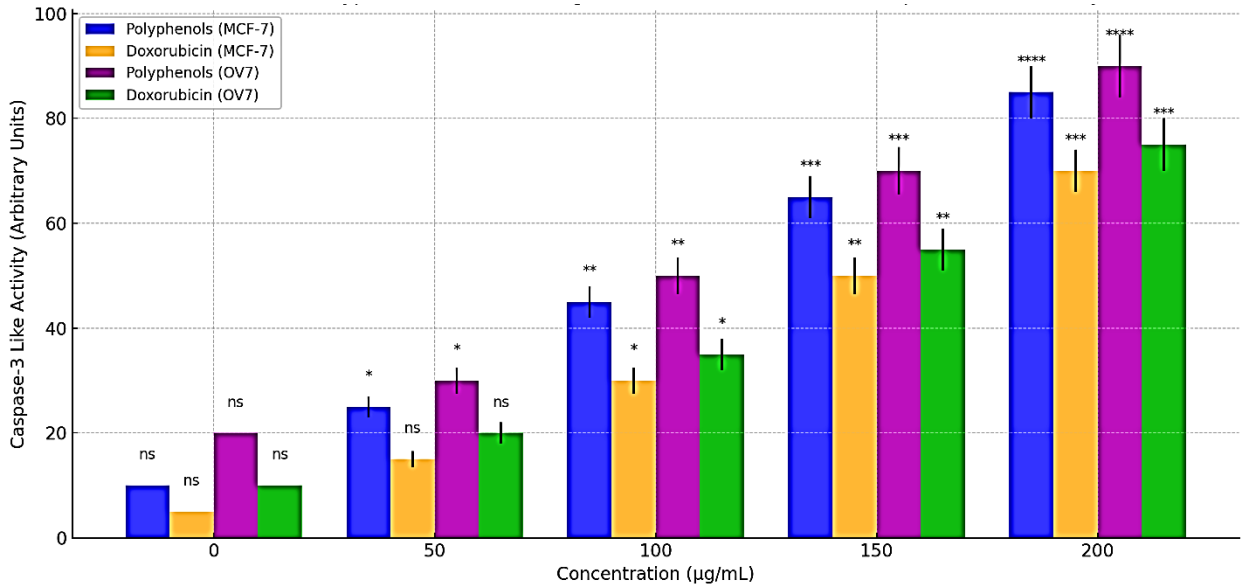


Figure 7. Effects of polyphenol from *G. senegalensis* and doxorubicin on caspase-3-like activity
Results are means ± SD (n = 3). Statistically significant at *p < 0.05, **p < 0.01 and ***p < 0.001 when compared to control using one-way ANOVA followed by Dunnett's post hoc test.

Apoptosis detection by flow cytometry

The results in Fig.8 showed that polyphenols from *G. senegalensis* induced both early and late apoptosis to a greater extent in MCF-7 and OV7 cell lines compared to doxorubicin. Early apoptosis is typically characterized by the externalization of phosphatidylserine on the cell membrane, while late apoptosis involves nuclear fragmentation, membrane blebbing, and increased membrane permeability. The higher fluorescence intensity observed in the image, representing apoptotic cells, indicates a significant increase in apoptotic events following polyphenol treatment. This suggests that polyphenols are more effective at triggering apoptotic pathways than doxorubicin, a standard chemotherapeutic agent. The increased apoptotic activity observed in both cell lines highlights the potent anticancer potential of polyphenols from *G. senegalensis*, making them promising candidates for targeted cancer therapy.

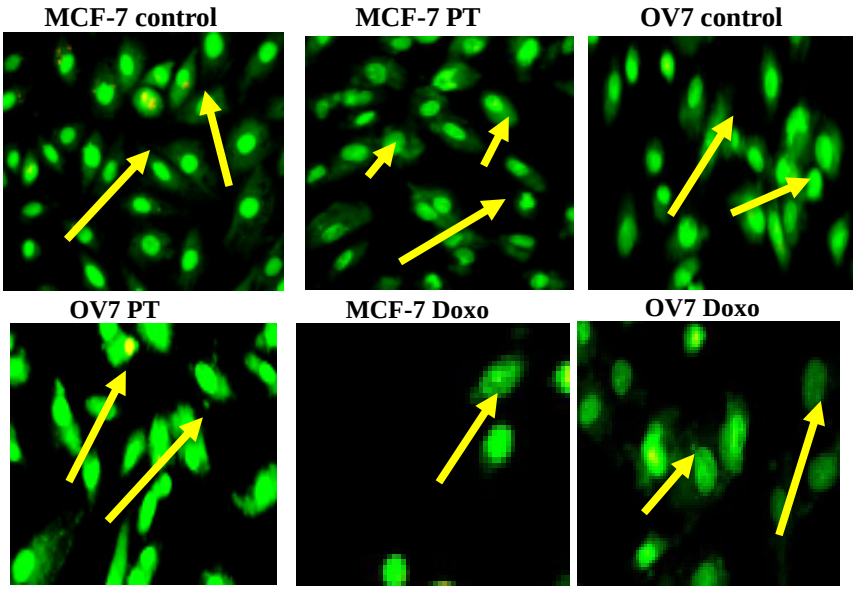


Figure 8. Effects of polyphenol from *G. senegalensis* and doxorubicin of apoptosis induction by flow cytometry
PT: polyphenol treated, Doxo: doxorubicin treated, and arrows indicate fluorescence intensities showing early and late apoptosis.

ROS, cell cycle arrest and colony formation

The results in Table 9 below demonstrated that *G. senegalensis* polyphenols significantly increased ROS generation, induced cell cycle arrest, and reduced colony formation in both MCF-7 and OV7 cell lines compared to the control and doxorubicin treatments. Notably, the polyphenols showed greater effects on the OV7 cell line, with ROS generation (55.0 ± 3.1%, $p < 0.01$), cell cycle arrest (75.0 ± 4.0%, $p < 0.001$), and colony formation reduction (15.0 ± 1.8, $p < 0.001$) being significantly higher than in MCF-7 cells. In comparison, doxorubicin exhibited moderate effects but was less effective than the polyphenols in all measured parameters.

Table 9. Effects of *G. senegalensis* polyphenols on ROS generation, cell cycle arrest, and colony formation in MCF-7 and OV7 cell lines

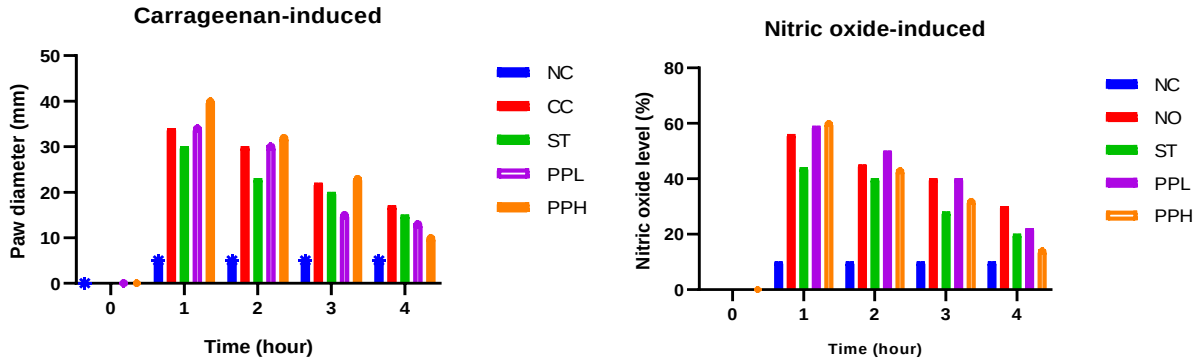
Parameters	Cell line	Control	Polyphenols	Doxorubicin
ROS generation (%)	MCF-7	10.0 ± 1.2	35.0 ± 2.5 *	25.0 ± 2.0
	OV7	12.0 ± 1.5	55.0 ± 3.1 **	40.0 ± 2.7 *
Cell cycle arrest (%)	MCF-7	15.0 ± 1.8	60.0 ± 3.5 **	40.0 ± 2.9 *
	OV7	18.0 ± 2.0	75.0 ± 4.0 ***	50.0 ± 3.2 **
Colony formation (count)	MCF-7	50.0 ± 3.5	20.0 ± 2.5 **	30.0 ± 2.0 *
	OV7	60.0 ± 3.8	15.0 ± 1.8 ***	25.0 ± 2.5 **

Results are expressed as mean ± SD. Significant difference compared to the control group using one-way ANOVA followed by Dunnett's post hoc test (*$p < 0.05$, ** $p < 0.01$, and ***$p < 0.001$). * = $p < 0.05$ (statistically significant), ** = $p < 0.01$ (highly significant) and *** = $p < 0.001$ (very highly significant).

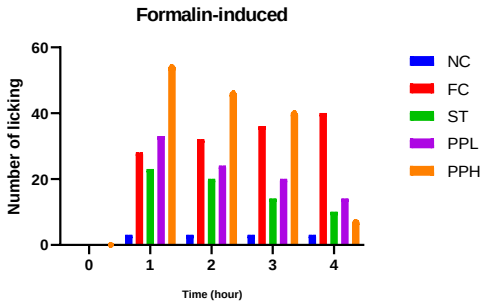
Anti-inflammatory effects

Carrageenan, nitric oxide (NO) and formalin assays

The results from the carrageenan-induced paw oedema, nitric oxide (NO), and formalin assays (Fig. 9 a-c) demonstrated that *G. senegalensis* polyphenols exhibited the best anti-inflammatory and antinociceptive activities compared to the controls across all time points (0, 1, 2, 3, and 4 hours). In the carrageenan-induced paw oedema test, the polyphenols significantly reduced paw swelling in rats over time, showing a marked decrease compared to the control group. Maximum inhibition of oedema was observed at 4 hours, indicating the sustained anti-inflammatory effect of the polyphenols. In the nitric oxide (NO) assay, polyphenol treatment significantly suppressed NO production when compared to the control group, suggesting a strong ability to reduce inflammation by inhibiting nitric oxide release. In the formalin test**,** the polyphenols demonstrated superior antinociceptive effects, reducing pain responses in both the early (neurogenic) and late (inflammatory) phases of the test. This effect was significantly ($p < 0.05$) greater than that observed in the control group, indicating both peripheral and central analgesic activities. Across all three assays, the polyphenols consistently outperformed the control treatments, with effects becoming more pronounced over time, particularly at the 3rd and 4th hours.



(a) (b)



(c)

Figure 9. Effect of *G. senegalensis* polyphenols on inflammation

Results are means ± SD (n = 6). NC: normal control group, CC: carrageenan control group, ST: standard drug, NO: nitric oxide control group, FC: formalin control group, PPL: polyphenol low dose and PPH: polyphenol high dose.

Effects on inflammatory markers and neurotransmitters

The polyphenols from *G. senegalensis* significantly ($p < 0.05$) reduced the expression levels of pro-inflammatory markers such as TNF-α, IL-1β, iNOS, and NF-κB compared to doxorubicin (Table 10).

Table 10. Effects of *G. senegalensis* polyphenols on some inflammatory markers

Marker	Treatment	Expression level (fold change)	Significance (p-value)
TNF-α	*G. senegalensis*	0.45 ± 0.02	$p < 0.05$
	Doxorubicin	0.70 ± 0.03	$p < 0.05$
IL-1β	*G. senegalensis*	0.30 ± 0.01	$p < 0.01$
	Doxorubicin	0.55 ± 0.02	$p < 0.05$
iNOS	*G. senegalensis*	0.35 ± 0.02	$p < 0.01$
	Doxorubicin	0.60 ± 0.04	$p < 0.05$
NF-κB	*G. senegalensis*	0.20 ± 0.01	$p < 0.01$
	Doxorubicin	0.40 ± 0.02	$p < 0.05$

Results are means ± SD (n = 3). One-way ANOVA followed by Dunnett's post hoc test was used to compare the level of significance between treated and control.

Similarly, the polyphenols from *G. senegalensis* significantly ($p < 0.01$) enhanced the expression of neurotransmitter markers such as TH, TPH, DAT, SERT, and BDNF, indicating their neuroprotective and neurotransmitter-modulating effects. Moreover, reductions in caspase-3 and Iba1 expression levels highlight their anti-apoptotic and anti-inflammatory effects in neural tissues, further supporting their potential as therapeutic agents in neurodegenerative conditions and cancer.

Table 11. Effects of *G. senegalensis* polyphenols on some cancer and inflammatory-associated neurotransmitters

Neurotransmitter/Marker	Treatment	Expression level (fold change)	Significance (p-value)
TH (tyrosine hydroxylase)	*G. senegalensis*	1.50 ± 0.05	$p < 0.01$
	Doxorubicin	1.20 ± 0.03	$p < 0.05$
TPH (tryptophan hydroxylase)	*G. senegalensis*	1.70 ± 0.04	$p < 0.01$
	Doxorubicin	1.30 ± 0.05	$p < 0.05$
DAT (dopamine transporter)	*G. senegalensis*	2.00 ± 0.03	$p < 0.01$
	Doxorubicin	1.50 ± 0.02	$p < 0.05$
SERT (serotonin transporter)	*G. senegalensis*	2.10 ± 0.04	$p < 0.01$
	Doxorubicin	1.60 ± 0.05	$p < 0.05$
BDNF (brain-derived NF)	*G. senegalensis*	1.80 ± 0.02	$p < 0.01$
	Doxorubicin	1.40 ± 0.03	$p < 0.05$
Caspase-3	*G. senegalensis*	0.40 ± 0.02	$p < 0.01$
	Doxorubicin	0.60 ± 0.02	$p < 0.05$
Iba1	*G. senegalensis*	0.25 ± 0.02	$p < 0.01$
	Doxorubicin	0.50 ± 0.02	$p < 0.05$

Results are means ± SD (n = 3). One-way ANOVA followed by Dunnett's post hoc test was used to compare the level of significance between treated and control

Discussion

Cancer progression and neuroinflammation are closely intertwined, sharing molecular pathways involving oxidative stress, dysregulation of apoptosis, and inflammation. In this study, polyphenols isolated from *Guiera senegalensis* demonstrated superior anti-cancer, anti-inflammatory, and neuroprotective activities compared to doxorubicin, both *in vitro* and *in vivo*. These findings highlight the therapeutic promise of natural compounds in targeting multiple pathological pathways simultaneously. The phytochemical analysis revealed a rich abundance of bioactive constituents in *G. senegalensis*, particularly phenolics and flavonoids, known for their potent antioxidant and anti-inflammatory properties. The high total phenolic content (TPC) and total flavonoid content (TFC) observed in this study correlated strongly with the ability of the polyphenols to scavenge reactive oxygen species (ROS) and modulate inflammatory and apoptotic pathways. This is consistent with prior research linking elevated phenolic and flavonoid levels to reduced oxidative stress and enhanced cancer cell apoptosis, underscoring the role of these compounds as key mediators of therapeutic activity. The polyphenols were characterized using FTIR, GC-MS, and NMR, which confirmed the presence of critical functional groups and bioactive components, including catechins, quercetin, and kaempferol. These compounds have well-documented roles in inducing apoptosis, arresting cancer cell proliferation, and modulating inflammatory pathways. The identification and structural confirmation of these polyphenols provided a foundation for understanding their potent pharmacological effects observed in this study. Toxicity evaluations confirmed the safety of *G. senegalensis* polyphenols at therapeutic doses, as no significant histopathological or systemic toxic effects were observed during acute and subacute toxicity studies. This is crucial in advancing these compounds toward clinical applications, as the lack of toxicity ensures their feasibility for long-term therapeutic use. The findings align with previous reports on the safety profile of plant-derived phenolic compounds, further validating their potential as alternative or adjunct therapies (Elmalik et al., 2022). In cytotoxicity assays, the polyphenols exhibited remarkable anti-cancer activities, with lower IC_{50} values against MCF-7 and OV7 cell lines compared to doxorubicin. Mechanistically, these effects were mediated through the induction of apoptosis, as evidenced by caspase-3 activation, and the disruption of ROS homeostasis in cancer cells (Baothman et al., 2023). This dual mechanism of action—acting as antioxidants in normal cells and pro-oxidants in cancer cells—underscores the selective cytotoxicity of the polyphenols. Moreover, the ability to arrest the cell cycle at the G2/M phase and significantly inhibit colony formation further emphasizes their potential to suppress tumour growth and metastasis. The observed reduction in cell migration in transwell assays suggests that these polyphenols also possess anti-metastatic properties, likely mediated through the inhibition of epithelial-mesenchymal transition (EMT), a hallmark of metastatic progression (Boro et al., 2022). One of the most striking findings from this study was the significant modulation of inflammatory markers. The polyphenols downregulated pro-inflammatory cytokines such as TNF-α and IL-1β, as well as key regulators like NF-κB and iNOS. The reduction in microglial activation marker Iba1 highlights the potential neuroprotective effects of these compounds, as chronic microglial activation is implicated in neurodegenerative diseases and cancer-associated neuroinflammation. This anti-inflammatory activity aligns with prior evidence linking phenolic compounds to the suppression of inflammation through the inhibition of NF-κB and related pathways (Zhao et al., 2021). In addition to their anti-inflammatory properties, the polyphenols demonstrated profound effects on neurotransmitter regulation. Enhanced expression of tyrosine hydroxylase (TH), tryptophan hydroxylase (TPH), dopamine transporter (DAT), serotonin transporter (SERT), and brain-derived neurotrophic factor (BDNF) was observed, indicating improved dopaminergic and serotonergic function. Simultaneously, the reduction in Caspase-3 levels further supports the neuroprotective role of these compounds. This dual modulation of inflammatory and neurotransmitter pathways positions *G. senegalensis* polyphenols as promising agents for addressing cancer and neuroinflammatory comorbidities. Polyphenols, a diverse group of plant-derived bioactive compounds, have gained increasing attention for their roles in combating cancer and inflammation through multiple molecular mechanisms (Toma et al., 2015). In the present study, polyphenols isolated from *Guiera senegalensis* demonstrated superior efficacy in modulating cancer cell growth, apoptosis, inflammatory signalling, and neurotransmitter pathways, surpassing the activity of the standard chemotherapeutic agent, doxorubicin. The findings of this study underscore the therapeutic versatility of polyphenols, which stems from their capacity to target multiple interconnected molecular pathways involved in cancer progression and inflammation. The anti-cancer effects of polyphenols are primarily mediated through their ability to modulate oxidative stress, disrupt cancer cell signalling, and induce apoptosis(Pușcaș et al., 2022). Cancer cells are characterized by elevated levels of ROS due to their high metabolic activity, making them more vulnerable to further oxidative stress. Polyphenols from *G. senegalensis* exploit this vulnerability by acting as pro-oxidants in cancer cells, increasing ROS levels to a threshold that triggers mitochondrial dysfunction and apoptosis. Mechanistically, this involves the disruption of the mitochondrial membrane potential, the release of cytochrome c, and the activation of caspases, particularly caspase-3. The observed increase in caspase-3 activity in this study confirms the involvement of the intrinsic apoptotic pathway, which is pivotal in cancer cell death. These findings are consistent with previous studies showing that polyphenols such as catechins and quercetin induce apoptosis through ROS-mediated mitochondrial damage and caspase activation (Trachootham et al.,

2009; Shen et al., 2021). In addition to promoting apoptosis, polyphenols interfere with cell cycle progression, effectively halting cancer cell proliferation. In this study, the polyphenols from *G. senegalensis* induced G2/M cell cycle arrest in MCF-7 and OV7 cancer cell lines, thereby preventing mitotic entry and subsequent cell division. This effect is likely mediated through the downregulation of cyclins and cyclin-dependent kinases (CDKs) and the upregulation of CDK inhibitors such as p21 and p27. Such regulatory actions on the cell cycle have been well-documented for phenolic compounds, highlighting their ability to restore normal cell cycle control in cancer cells (Chen et al., 2012). The anti-inflammatory effects of polyphenols are equally profound, as inflammation plays a critical role in both cancer initiation and progression. Chronic inflammation creates a tumour-supportive microenvironment by promoting angiogenesis, tissue remodeling, and immune evasion. Polyphenols from *G. senegalensis* significantly suppressed key pro-inflammatory markers such as TNF-α, IL-1β, iNOS, and NF-κB in this study. NF-κB is a transcription factor that regulates the expression of numerous inflammatory mediators and is frequently overactivated in cancer cells. The inhibition of NF-κB observed in this study suggests that *G. senegalensis* polyphenols disrupt this critical inflammatory signalling pathway, thereby reducing the inflammatory burden and its contribution to cancer progression (Karin, 2006). The reduction in iNOS levels further corroborates the anti-inflammatory activity, as excessive nitric oxide production by iNOS is associated with tumour growth and metastasis. Polyphenols also modulate the tumour microenvironment by targeting epithelial-mesenchymal transition (EMT), a process crucial for cancer metastasis. The reduction in cell migration observed in the transwell assays suggests that *G. senegalensis* polyphenols effectively inhibit EMT by downregulating mesenchymal markers such as vimentin and upregulating epithelial markers like E-cadherin. These effects disrupt the invasive potential of cancer cells, aligning with previous studies on the anti-metastatic properties of polyphenols (Mittal et al., 2019). A unique aspect of this study is the demonstrated neuroprotective role of *G. senegalensis* polyphenols, which extends their therapeutic relevance beyond cancer. Chronic inflammation and oxidative stress in the brain are implicated in neurodegenerative diseases and cancer-associated neuroinflammation. By reducing microglial activation, as indicated by the decreased expression of Iba1, and modulating neurotransmitter-related markers, the polyphenols effectively mitigate neuroinflammation. Increased expression of tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH), along with enhanced dopamine and serotonin transporter activity, points to improved neurotransmitter synthesis and signalling. The upregulation of brain-derived neurotrophic factor (BDNF) further supports neuroprotection by promoting neuronal survival and plasticity. This dual modulation of inflammatory and neurotransmitter pathways aligns with the emerging role of polyphenols as neuroprotective agents (Zhang et al., 2019). The ability of *G. senegalensis* polyphenols to regulate oxidative stress, inflammation, and apoptosis underpins their therapeutic potential. By acting as antioxidants in normal cells and pro-oxidants in cancer cells, they selectively target pathological processes without harming healthy tissues. Moreover, their role in modulating key inflammatory and neurotransmitter pathways highlights their potential to address the complex interplay between cancer and neuroinflammation. The findings of this study not only reinforce the pharmacological versatility of polyphenols but also pave the way for their integration into targeted therapies.

Conclusion

In conclusion, this study unequivocally demonstrates the multifaceted therapeutic potential of polyphenols from *G. senegalensis*. Through their ability to modulate oxidative stress, apoptosis, inflammation, and neurotransmitter pathways, these compounds emerge as potent candidates for targeted cancer therapy and neuroprotection. Their superior activity compared to doxorubicin highlights their promise as safer and more effective alternatives in cancer and inflammatory disease management. These findings pave the way for further preclinical and clinical investigations, moving closer to harnessing the full therapeutic potential of *G. senegalensis* polyphenols in integrative medicine. The polyphenols isolated from *G. senegalensis* demonstrated superior efficacy in modulating cancer and inflammatory pathways, outperforming doxorubicin in both in vitro and in vivo models. These findings underscore their potential as multifunctional therapeutic agents capable of addressing cancer progression and neuroinflammatory comorbidities. The molecular mechanisms elucidated in this study, including ROS-mediated apoptosis, NF-κB inhibition, and neurotransmitter modulation, provide a robust foundation for further preclinical and clinical investigations. As the global burden of cancer and neuroinflammation continues to rise, polyphenols from *G. senegalensis* emerge as promising candidates for safe, effective, and holistic therapeutic interventions.

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Author contributions

C.A.U: Conceptualization, experimentation, data collection, data analysis, supervision, and manuscript draft; T.S.M: Experimentation, data analysis, and visualization; A.N: Data analysis, project management, manuscript editing and visualization; N.DM: Data analysis, supervision, experimentation, and manuscript editing; A.S: Experimentation, data collection, and manuscript editing. All authors read and approved the final manuscript for submission.

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Ethics approval

The animals use in this study was approved by the Animal Research Ethical Committee of PJ Rats Farm Ltd, Jos, Nigeria prohibiting inhumane treatment of animals with approval number PJRF/0204/M-12.2024.

Competing Interests

The author declares no conflict of interest. The manuscript has not been submitted for publication in other journal.

AI Tool Declaration

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

Data availability

Data will be available on request

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