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Potential Cytotoxic Isolates of *Spigelia anthelmia* Linn. against T47D and WiDr Cancer Cells

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Abstract

Natural products continue to provide valuable leads for anticancer drug discovery, yet limited studies have addressed the bioactive constituents of *Spigelia anthelmia* Linn. This research aimed to isolate and characterize cytotoxic metabolites of *S. anthelmia* using a bioassay-guided isolation approach. Sequential extraction, vacuum liquid chromatography (VLC), and preparative thinlayer chromatography (PTLC) yielded a semi-purified fraction designated as Isolate A. Cytotoxicity was evaluated against T47D breast cancer and WiDr colon cancer cells using the MTT assay. Isolate A significantly reduced cell viability in a dose-dependent manner (ANOVA, $p < 0.05$), with IC_{50} values of 166.92 ± 5.10 $\mu\text{g/mL}$ (T47D) and 245.24 ± 6.25 $\mu\text{g/mL}$ (WiDr), whereas doxorubicin exhibited IC_{50} values of 40.05 ± 2.30 $\mu\text{g/mL}$ and 3.57 ± 0.45 $\mu\text{g/mL}$, respectively. Flow cytometry with Annexin V-FITC/PI staining revealed a visible shift of cell populations toward early and late apoptotic

quadrants, indicating apoptosis as the predominant mechanism of cell death. Spectroscopic characterization by FTIR, LC–MS, and NMR suggested that Isolate A is a phenolic/alkaloid-type compound (m/z 218 $[M+H]^+$) containing hydroxyl, carbonyl, and heteroaromatic groups. Collectively, these findings demonstrate that *S. anthelmia* contains apoptosis-inducing metabolites with measurable cytotoxicity, supporting its potential as a natural source of anticancer leads and warranting further purification and mechanistic investigation. To our knowledge, this is the first study to combine bioassay-guided isolation, apoptosis profiling, and spectroscopic characterisation of *S. anthelmia* metabolites in T47D and WiDr cancer cells. © 2025 Taupik et al.

Author keywords

Apoptosis; Bioassay-guided isolation; Cytotoxicity; Phenolic alkaloids; Spectroscopic characterization; *Spigelia anthelmia*

Indexed keywords

EMTREE drug terms

alkaloid; antineoplastic agent; doxorubicin; phytochemical; plant extract; protein bcl 2

EMTREE medical terms

apoptosis; Article; bioassay; breast cancer; cancer cell; carbon nuclear magnetic resonance; cell viability; colon cancer cell line; cytotoxicity; female; flow cytometry; fraction absorbed; human; human cell; IC₅₀; liquid chromatography; liquid chromatography-mass spectrometry; metabolite; MTT assay; proton nuclear magnetic resonance; spectroscopy; T-47D cell line; thin layer chromatography; WiDr cell line

Device trade names

Commercial names given to devices, used for branding and differentiation in the market, commonly referenced in scientific and clinical research.

Tradename	Country	Manufacturer
FTIR 100	United States	Perkin Elmer

Chemicals and CAS Registry Numbers

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Unique identifiers assigned by the Chemical Abstracts Service (CAS) to ensure accurate identification and tracking of chemicals across scientific literature.

doxorubicin	23214-92-8, 25316-40-9
protein bcl 2	219306-68-0

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