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Original Research

Cytotoxic Activity of *Chromolaena odorata* Leaf Extract on WiDr Colon Cancer Cells

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ABSTRACT

Background: Colorectal cancer (CRC) remains the third most commonly diagnosed cancer and the second leading cause of cancer-related mortality worldwide. In 2020, CRC accounted for approximately 1.9 million new cases and over 935,000 deaths globally. Conventional chemotherapy is frequently limited by systemic toxicity and drug resistance, necessitating the identification of novel plant-derived bioactive compounds. *Chromolaena odorata* (Asteraceae), a tropical medicinal plant, has demonstrated pharmacological properties including wound healing and anti-inflammatory activity; however, its antiproliferative potential against colorectal cancer cells remains insufficiently explored. **Objective:** This study aimed to evaluate the cytotoxic and antiproliferative effects of *C. odorata* n-hexane leaf extract on WiDr human colorectal adenocarcinoma cells using an in vitro MTT assay. **Methods:** Dried leaves of *C. odorata* were extracted via n-hexane maceration. WiDr cells were cultured in DMEM supplemented with 10% FBS and antibiotics. Cells were treated with graded extract concentrations (62.5–1000 µg/mL) for 24 hours in triplicate. Cell viability was assessed by the MTT assay and the half-maximal inhibitory concentration (IC_{50}) was determined via nonlinear regression analysis. **Results:** The extract exhibited concentration-dependent cytotoxicity against WiDr cells. The highest mean inhibition rate (94.3 ± 10.7%) was observed at 1000 µg/mL. The calculated IC_{50} value was 149.09 ± 20.91 µg/mL, indicating moderate cytotoxic potency consistent with the National Cancer Institute (NCI) classification for biologically active crude extracts (IC_{50} < 200 µg/mL). **Conclusion:** *Chromolaena odorata* n-hexane leaf extract demonstrates significant in vitro antiproliferative activity against WiDr colorectal cancer cells. The cytotoxic activity likely involves lipophilic bioactive compounds, including terpenoids. These findings support further bioassay-guided fractionation, mechanistic investigation, and in vivo validation as potential candidates for anticancer drug development.

Keywords: Colorectal cancer; WiDr cells; *Chromolaena odorata*; cytotoxicity; terpenoids.

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1. INTRODUCTION

Colorectal cancer (CRC) ranks third among the most commonly diagnosed malignancies worldwide and represents the second leading cause of cancer-related mortality (Sung et al., 2021). In 2020 alone, CRC accounted for approximately 1.9 million new cases and over 935,000 deaths globally. The incidence continues to rise, particularly in low- and middle-income countries, including those in Southeast Asia, where rapid urbanization and westernized dietary patterns have significantly altered behavioral risk factors. Increased consumption of high-fat and low-fiber diets, smoking prevalence, alcohol intake, obesity, and sedentary behavior are strongly associated with colorectal carcinogenesis (Cho et al., 2019; Xi & Xu, 2021). In Indonesia, CRC ranks third in overall cancer incidence, with South Sulawesi Province similarly classified among the provinces with the highest CRC burden (Kementerian Kesehatan RI, 2024).

At the molecular level, colorectal carcinogenesis involves multistep genetic and epigenetic alterations that disrupt key signaling pathways regulating cell proliferation, apoptosis, and differentiation. Among the most frequently dysregulated pathways are Wnt/β-catenin, PI3K/Akt/mTOR, MAPK, and p53 tumor suppressor signaling. These molecular complexities partly explain therapeutic resistance and disease recurrence (Xi & Xu, 2021).

Conventional therapeutic approaches for CRC include surgical resection, chemotherapy, radiotherapy, and targeted therapy. Although these strategies have improved survival rates, systemic chemotherapy—particularly regimens involving 5-fluorouracil, oxaliplatin, or irinotecan—is often associated with severe adverse effects such as myelosuppression, gastrointestinal toxicity, neuropathy, hepatotoxicity, and the development of multidrug resistance (Darmawan et al., 2019). Therefore, there is an urgent need to explore novel therapeutic agents with effective and improved safety profiles.

Natural products continue to play a pivotal role in anticancer drug discovery. Plant secondary metabolites—including terpenoids, alkaloids, tannins, phenolic acids, and flavonoids—have demonstrated diverse anticancer activities through mechanisms such as induction of apoptosis, cell cycle arrest, reactive oxygen species (ROS) modulation, inhibition of angiogenesis, and suppression of oncogenic signaling pathways (Mayanda & Gunawan, 2024).

Chromolaena odorata (family Asteraceae), locally known as kirinyuh, is a tropical medicinal plant widely distributed across Southeast Asia. Traditionally, it has been used for wound healing, anti-inflammatory treatment, and infection control (Putri et al., 2021; Ernawati & Jannah, 2021). Phytochemical analyses of its n-hexane leaf extract have revealed a predominance of lipophilic constituents, particularly terpenoids and other non-polar compounds, that may possess cytotoxic properties. While antimicrobial, antioxidant, and wound-healing activities of *C. odorata* have been reported, data regarding its antiproliferative effects against colorectal cancer cell lines remain limited.

The WiDr cell line, derived from human colorectal adenocarcinoma, is widely used as an in vitro model for anticancer screening due to its well-characterized molecular background (Noguchi et al., 1979). Despite the recognized pharmacological potential of *C. odorata*, there remains a lack of systematic investigation evaluating its cytotoxic activity specifically against WiDr cells. This study therefore aimed to determine the IC_{50} value of *C. odorata* n-hexane leaf extract against WiDr colorectal adenocarcinoma cells using the MTT assay, providing a foundation for subsequent mechanistic and translational investigations.

2. METHODS

2.1 Study Design

This was an in vitro experimental laboratory study conducted to evaluate the cytotoxic activity of *C. odorata* n-hexane leaf

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extract on WiDr human colorectal adenocarcinoma cells.

2.2 Plant Material and Extraction

Fresh leaves of *Chromolaena odorata* were collected, washed, and air-dried followed by oven-drying at 40C for three days. Dried leaves were powdered and macerated with n-hexane for 72 hours at room temperature with occasional stirring. The filtrate was collected and evaporated using a rotary evaporator at 45C to yield a concentrated n-hexane extract (Putry et al., 2021). The use of n-hexane as the extraction solvent preferentially recovers lipophilic constituents including terpenoids, sterols, and other non-polar compounds (Harborne, 1998; Huang et al., 2012).

2.3 Sample Preparation

A stock solution of 1000 g/mL was prepared by dissolving 10 mg of the n-hexane extract in 100 L dimethyl sulfoxide (DMSO) and 900 L DMEM culture medium. Serial dilutions in DMEM were subsequently performed to achieve final test concentrations of 1000, 500, 250, 125, and 62.5 g/mL. The final DMSO concentration in all treatment wells did not exceed 0.1% (v/v), a concentration confirmed to be non-toxic to WiDr cells.

2.4 Cell Culture

The WiDr human colorectal adenocarcinoma cell line (Noguchi et al., 1979; Chen et al., 1987) was cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 1% amphotericin B. Cells were maintained at 37°C in a humidified CO_2 incubator with 5% CO_2. Cells were sub-cultured and harvested for cytotoxicity testing upon reaching 70–80% confluency using 0.25% trypsin-EDTA.

2.5 Cytotoxicity Assay (MTT Assay)

WiDr cells were seeded into 96-well plates at a density of 5×10^3 cells/well and incubated for 24 hours to allow adherence. The culture medium was then aspirated and replaced with 100 µL of each extract concentration (62.5, 125, 250, 500, and 1000 µg/mL) in triplicate. Untreated cells (negative control, culture medium with 0.1% DMSO) were included in each plate. Plates were incubated for 24 hours at 37°C with 5% CO_2.

After the treatment period, 10 µL of MTT reagent (5 mg/mL in phosphate-buffered saline) was added to each well and incubated for 4 hours at 37°C, following the standard protocol (Mosmann, 1983). The medium was carefully removed and 100 µL of DMSO was added to dissolve the resulting formazan crystals. Plates were incubated overnight at room temperature in the dark. Absorbance was measured at 570 nm using a microplate reader. The percentage of cell inhibition was calculated using the formula:

$$\% \, Inhibition = [(OD\text{control} - OD\text{treatment}) / OD\text{control}] \times 100$$

2.6 IC_{50} Determination and Statistical Analysis

The IC_{50} value was determined from the dose–response curve using nonlinear regression analysis (GraphPad Prism, four-parameter logistic model). Data were expressed as mean ± standard deviation (SD) of three independent replicates. IC_{50} values from each independent experiment were used to calculate the mean IC_{50} and its SD.

3. RESULTS

3.1 Cytotoxic Activity of *C. odorata* n-Hexane Extract Against WiDr Cells

The n-hexane leaf extract of *C. odorata* exhibited concentration-dependent cytotoxicity against WiDr colorectal adenocarcinoma cells. Table 1 presents the percentage inhibition values across all tested concentrations.

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Table 1. Percentage inhibition of WiDr cells treated with *Chromolaena odorata* n-hexane leaf extract for 24 hours.

Concentration (µg/mL)	Replicate I (%)	Replicate II (%)	Replicate III (%)	Mean ± SD (%)
1000	82	100	101*	**94.3 ± 10.7**
500	92	92	87	**90.3 ± 2.9**
250	82	78	77	**79.0 ± 2.6**
125	39	48	36	**41.0 ± 6.2**
62.5	15	12	12	**13.0 ± 1.7**

**Note: Replicate III at 1000 g/mL yielded a raw inhibition value of 101%, likely attributable to minor pipetting variability or optical interference at high extract concentrations. This value was retained in the mean calculation for transparency.*

A progressive increase in inhibition was observed across the tested concentration range. Inhibition rose from 13.0 ± 1.7% at 62.5 µg/mL to a maximum of 94.3 ± 10.7% at 1000 µg/mL. A notable inflection was observed between 125 µg/mL (41.0 ± 6.2%) and 250 µg/mL (79.0 ± 2.6%), suggesting that 50% inhibition occurs within this range.

3.2 IC_{50} Value

Nonlinear regression analysis of the dose–response data yielded an IC_{50} value of **149.09 ± 20.91 µg/mL**. The dose–response curve is illustrated in Figure 1. According to the NCI classification criteria, crude plant extracts with IC_{50} values below 200 µg/mL are considered biologically active (Suffness & Pezzuto, 1990), classifying this extract as moderately cytotoxic.

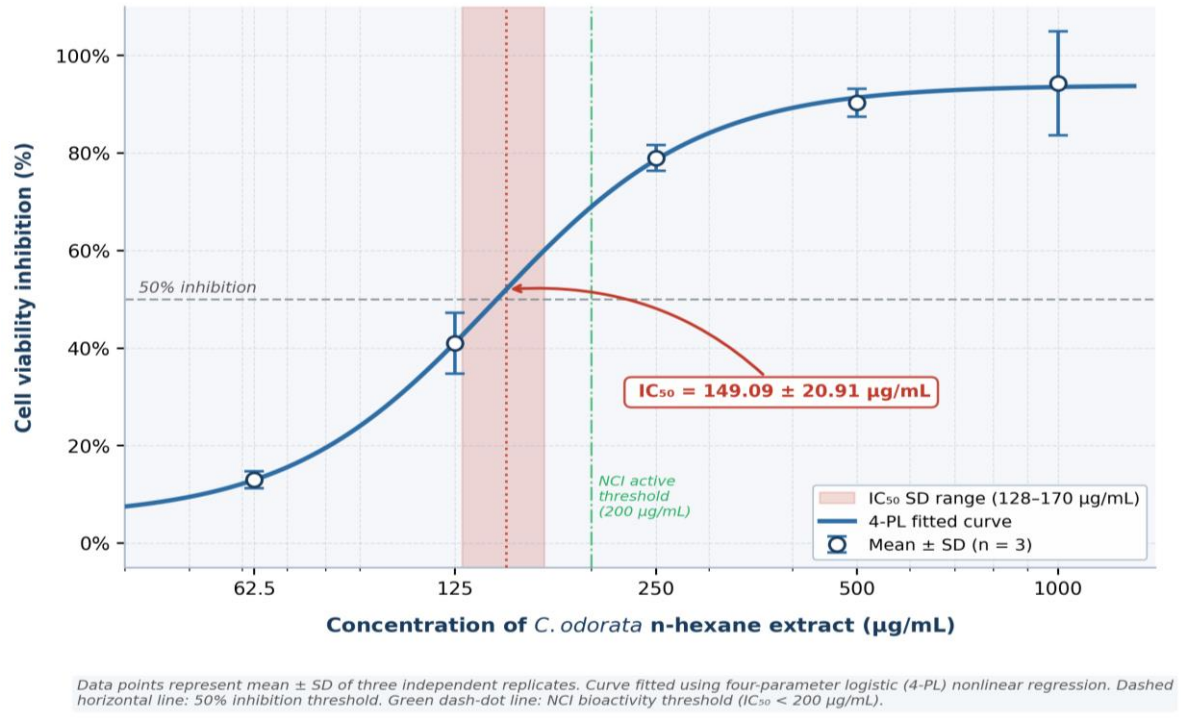

Figure 1. Dose–response curve of *Chromolaena odorata* n-hexane leaf extract against WiDr cells. IC_{50} = 149.09 ± 20.91 µg/mL.

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4. DISCUSSION

The present study demonstrates that the n-hexane leaf extract of *Chromolaena odorata* exerts significant concentration-dependent cytotoxic effects against WiDr colorectal cancer cells, with an IC_{50} of 149.09 ± 20.91 µg/mL. Based on the NCI classification criterion for crude extracts (IC_{50} < 200 µg/mL = biologically active; Suffness & Pezzuto, 1990), this result indicates moderate cytotoxic potency consistent with a primary screening finding.

The progressive increase in inhibition with rising extract concentrations confirms a classic dose–dependent cytotoxic response. The steepest rise in inhibition between 125 µg/mL (41.0%) and 250 µg/mL (79.0%) suggests a threshold-dependent mechanism in which bioactive constituents must accumulate to sufficient intracellular concentrations to trigger cytotoxic cascades. Similar dose–response patterns have been reported for crude plant extracts against colorectal cancer cell lines, with IC_{50} values typically ranging from 100 to 300 µg/mL (Noguchi et al., 1979).

Extraction with n-hexane preferentially recovers non-polar constituents; therefore, the cytotoxic activity observed in this study is most likely attributable to lipophilic compounds, particularly **terpenoids** and sterols, rather than polar phenolics or flavonoids. Terpenoids isolated from *Chromolaena odorata* have been reported to exhibit cytotoxic and pro-apoptotic properties. Several sesquiterpene lactones and diterpenes from related Asteraceae species have demonstrated activity against colon cancer models through induction of apoptosis and inhibition of cell proliferation.

Although mechanistic evaluation was beyond the scope of this study, potential mechanisms plausibly associated with terpenoid-mediated cytotoxicity include: (1) disruption of mitochondrial membrane integrity leading to caspase activation; (2) inhibition of cell cycle progression; and (3) modulation of oncogenic signaling pathways relevant to CRC, such as PI3K/Akt or Wnt/β-catenin. These hypotheses warrant systematic validation in future studies incorporating apoptosis assays, cell cycle analysis, and molecular pathway profiling.

The IC_{50} value reported here (149.09 µg/mL) is consistent with values reported for other plant-derived crude extracts tested against colorectal cancer lines. For instance, Aswad and Irma (2022) reported cytotoxic activity of *Brassica oleracea* var. botrytis extract against WiDr cells in a comparable range. Crude extracts generally yield higher IC_{50} values than purified fractions due to the diluting effect of inactive components. Bioassay-guided fractionation and isolation of active constituents from *C. odorata* are therefore expected to yield substantially lower IC_{50} values and more potent activity.

Several limitations of this study should be acknowledged. First, a standard positive control (e.g., doxorubicin or 5-fluorouracil) was not included in this screening experiment, limiting direct comparative potency assessment. Second, the study evaluated only a single colorectal cancer cell line (WiDr); evaluation against additional CRC lines and normal colon epithelial cells is needed to determine selectivity. Third, mechanistic assays (apoptosis, ROS, cell cycle analysis, Western blot) were not performed. Fourth, the in vitro findings require in vivo validation. These limitations notwithstanding, the observed IC_{50} value provides a rigorous quantitative basis for prioritizing *C. odorata* for advanced preclinical investigation.

To advance the translational potential of *C. odorata*, future studies should include: (1) bioassay-guided fractionation and identification of active terpenoid constituents; (2) apoptosis assays (Annexin V/PI flow cytometry); (3) cell cycle distribution analysis; (4) intracellular ROS quantification; (5) Western blot analysis of apoptotic markers (Bax, Bcl-2, Caspase-3, p53); (6) selectivity index determination using normal colon epithelial cells; (7) combination studies with standard chemotherapeutic agents; and (8) in vivo xenograft tumor models.

5. CONCLUSION

This study demonstrates that the n-hexane leaf extract of *Chromolaena odorata* exhibits concentration-dependent cytotoxic activity against WiDr human colorectal adenocarcinoma cells, with an IC_{50} of 149.09 ± 20.91 µg/mL, indicative of moderate antiproliferative potency. The observed activity is likely associated with lipophilic phytochemicals, predominantly terpenoids,

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recoverable by n-hexane extraction. These findings provide a quantitative foundation for further phytochemical investigation, mechanistic characterization, and in vivo validation of *Chromolaena odorata* as a candidate source for novel adjunct or alternative agents in colorectal cancer management.

DECLARATION

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Conflict of Interest

The authors declare no conflict of interest.

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