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Cytotoxic and Antiproliferative Effects of *Gynura divaricata* Ethanolic Extract on HCT-116 Colorectal Cancer Cells In Vitro

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ABSTRACT

Background: Colorectal cancer remains one of the leading causes of cancer-related morbidity and mortality worldwide. The limitations of conventional treatments, such as chemotherapy and radiotherapy—including adverse side effects and resistance—have prompted the exploration of alternative therapies derived from natural products. **Objective:** This study aimed to evaluate the cytotoxic effects of *Gynura divaricata* ethanolic extract on HCT-116 colorectal cancer cells and assess its potential as a complementary anticancer agent. **Methods:** Dried *Gynura divaricata* leaves were extracted using ethanol through maceration. HCT-116 cells were cultured and exposed to various concentrations of the extract. Cell viability was assessed using the MTT assay, and the half-maximal inhibitory concentration (IC_{50}) was determined via linear regression analysis. **Results:** The *Gynura divaricata* extract significantly reduced HCT-116 cell viability in a dose-dependent manner. The calculated IC_{50} value was 62.09 µg/mL, indicating notable cytotoxic activity. **Conclusion:** The findings suggest that *Gynura divaricata* possesses promising anticancer potential and may serve as an alternative or adjunctive therapeutic option for colorectal cancer.

Keywords : Colorectal cancer, cytotoxicity, *Gynura divaricata*, HCT-116.

INTRODUCTION

Colorectal cancer is the one of the leading causes of cancer-related morbidity and mortality worldwide. From the Surveillance, Epidemiology, and End Results (SEER) database estimates, over 153.000 new colorectal cancers were diagnosed in 2023, accounting for 7,8% of all newly diagnosed cancers [1]. Men are more likely to develop colon cancer (53%) and at a younger age than women (68 vs. 72 years)[1][2]. Initial evaluation may involve barium enema or CT colonography, ultimately, a colonoscopy is required for a tissue diagnosis [1][3][4]. Colonoscopy should include multiple biopsies of the suspected lesion and tattooing of the peritumoral colon to facilitate intraoperative identification. CEA is the most commonly used tumor marker for colon cancer and should be measured at baseline. High CEA levels correlate with poor prognosis, and the CEA levels after treatment are useful in detecting disease recurrence [4].

Despite advancements in conventional treatment such as surgery, chemotherapy, and also radiotherapy [5][6]. The search for alternative and complementary therapy remains critical due to issues like drug resistance, side effect, and recurrence [5][7]. Among potential candidates, *Gynura divaricata*, a traditional medicinal plant, has emerged as a promising source of bioactive compounds with cytotoxic effects on cancer cells [8][9][10]. Gynura divaricata, belonging to the family

compositae, is widely used in traditional medicine for treating various elements [8][11]. Studies have shown that Gynura divaricata mainly contains alkaloids, flavonoids, terpenoids, polysaccharides, aliphatic compounds, and so on that exhibit anti-oxidant activity and free radical-scavenging capacity, which may contribute to their ability to inhibit cancer cell proliferation and induce apoptosis [10][11][12][13][14].

The HCT-116 cell line, derived recognized model for studying colorectal cancer biology and therapeutic interventions. Preliminary research has demonstrated that Gynura divaricata extacts exert moderate cytotoxity agains Hepaticullar carcinoma (HCC) cell line [8]. Additionally, its ability to suppress signaling pathway of Wnt/β-catenin[8] critical for cancer stem cell growth, it is potential in targeting colorectal cancer.

This study aims to explore the impact of *Gynura divaricata* on cytotoxicity in HCT-116 cells. By investigating its mechanisms of action and therapeutic potential. This study seeks to contribute to the growing body of evidence supporting the use of plant-based compounds in cancer treatment.

MATERIAL AND METHODS

Plant material

The *Gynura divaricata* herb are obtained from supplier in B2P2TOOT (Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional) in Tawangmangu, Central Java, Indonesia. Subsequently, the plant material is dried using cabinet dryer at 40 - 50°C.

Extraction procedure

The dried *Gynura divaricata* herb was first tested for water content to ensure compliance with the standards set by the Indonesian Herbal Pharmacopeia, 2nd Edition. After meeting the required specifications, the dried material was subjected to size reduction using a 60-mesh grinder to obtain a fine powder. The powdered herb was then extracted by maceration using ethanol as the solvent at a ratio of 1:10 (w/v) for three days. This process was followed by a re-maceration step to maximize the yield of bioactive compounds. The combined filtrates were concentrated using a vacuum evaporator at a temperature range of 40–50°C to obtain a viscous ethanol extract of *G. divaricata*.

Cell material

Colorectal cancer HCT116 cells were acquired from the European Collection of Authenticated Cell Cultures (EACC, 91091005).

Cell culture and treatment

The HCT116 cells were cultured and maintained in F12 Dulbecco's modified Eagle's medium (DMEM) (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, USA) and 1.5% Penicillin-streptomycin antibiotic solutions (Gibco, USA) at 37°C and 5% CO2. After the cultured cells reached 80% confluency, they were treated with CLE and PNE in serial dose.

Cell viability test

The MTT assay was utilized to assess cell viability with a minor adjustment. In summary, $5\,x\,10^3\ cell/well$ were plated in 96-well plate and kept at 37°C with 5% CO2 for 24 hours. After cells have 80% of confluency per well, cell ready for treatment. Following this, cell was exposed to combination of 0 – 500 µg/mL of *Gynura divaricata* extract for 24 hours. Cells that were not treated served as negative control. Post – treatment, cells were exposed to 0.5 mg/mL of MTT and further incubate for 4 hours. After incubation, 100 µL of DMSO was added in the well and then incubated for 15 minutes. Subsequently, the absorbance was determined using ELISA reader (Biorad iMark

TM Microplate Reader) at wavelength 595 nm. The absorbance readings were converted into percentages of cell viability by comparing the treated group with untreated group at specific time points.

IC50 value

The IC50 value was determined by performing linear regression between the concentration (x) and % cell viability (y). Percent cell viability can be measure by

$$\%Cell\ viability = \frac{mean\ value\ of\ the\ absorbance\ test\ item}{mean\ value\ of\ the\ absorbance\ negative\ control}\ x\ 100\% \quad (1).$$

After we found percent cell viability, used the value to made the standard curve with linear equation. The IC50 value can be found by

$$IC50 = \frac{50 - B}{A} \quad (2)$$

where A is slope or gradient of the curve and B is intercept of linear equation. The IC50 value, representing the concentration that inhibits 50% of cell proliferation. The experimental data for this research was obtained from three independent replication experiments.

RESULTS

Absorbance value

After measuring absorbance using ELISA reader, the untreated group showed the highest absorbance value (~1,05). In the group treated with 25 $\mu g/mL$ of *Gynura divaricata*, the absorbance value was reduced by approximately half (~0,57). Subsequent concentrations also showed decreasing trend, indicating that higher concentrations of *Gynura divaricata* resulted in progressively lower absorbance values.

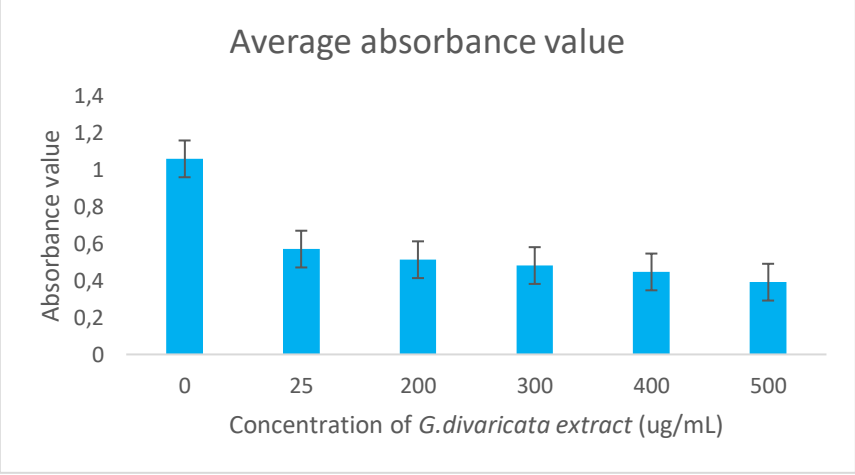

Figure 1. Absorbance value after treatment with Gynura divaricata multiple concentration

IC50 value

Using equation 1, we find % viability cell every concentration of treatment and we use every data to make the linear curve of regression. After that, using equation 2, we found that IC50 value is $62{,}09\ \mu g/mL$.

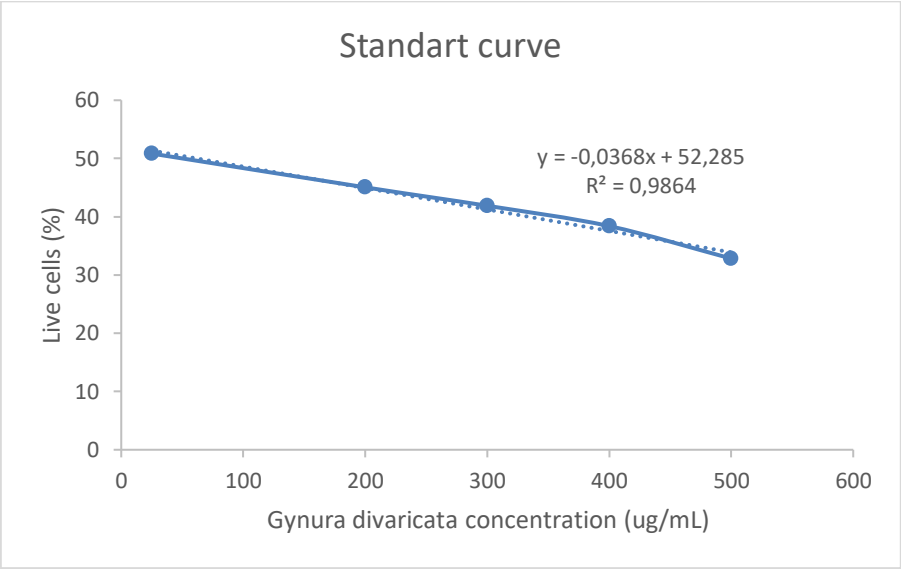

Figure 2. Standart curve for IC50

DISCUSSION

Based on the results of this study, the absorbance value decreased with increasing concentrations of *G. divaricata* extract in the treatment groups. This suggests that the extract reduces cell viability, possibly by inhibiting cell proliferation or inducing apoptosis. These findings are consistent with the Beer-Lambert Law in spectrophotometric analysis, which states that absorbance is directly proportional to the concentration of a solute in a solution [15,16]. In other words, a decrease in absorbance may indicate a reduction in the number of viability of cells. This relationship is further supported by the IC50 calculation shown in Figure 2, which demonstrates that higher concentrations of the extract correspond to a lower percentage of cell viability.

Several bioactive compounds in *G. divaricata*, such as flavonoids kaempferol, and quercetin, have demonstrated antiproliferative activity and can induce apoptosis in breast cancer cells, as shown in studies involving T47D and MCF-7 cell [13,16]. These compounds have been shown to induce apoptosis through both intrinsic (mitochondrial) and extrinsic pathways. Mechanistically, quercetin and kaempferol can upregulate pro-apoptotic proteins such as Bax and caspase-3, while downregulating anti-apoptotic proteins such as Bcl-2 [17]. This shift in the Bcl-2/Bax ratio facilitates mitochondrial membrane permeabilization, leading to cytochrome c release and activation of the caspase cascade. In addition, *G. divaricata* has been reported to cause cell cycle arrest, particularly at the G0/G1 and G2/M phases, through modulation of key regulatory proteins such as cyclin D1, CDK4, and p21. Arresting the cell cycle at these checkpoints prevents the replication of damaged DNA and halts cancer cell proliferation [18].

A particularly notable mechanism is the inhibition of the Wnt/β-catenin signaling pathway, a critical driver of cancer stem cell (CSC) self-renewal, tumor growth, and recurrence. *G. divaricata* extract has been shown to suppress the expression of β-catenin, thereby disrupting downstream

transcription of oncogenes such as c-Myc and cyclin D1 [8,19]. Additionally, it reduces the expression of CSC markers such as CD44, CD133, and EpCAM, which are associated with tumor aggressiveness and resistance to conventional therapies. Other compounds in the extract, such as polysaccharides, have also demonstrated immunomodulatory and antitumor effects. These include the activation of macrophages and natural killer (NK) cells, as well as the inhibition of angiogenesis and tumor metastasis [20].

The aqueous leaf extract of *Gynura divaricata* also exhibit cytotoxic activity against T47D cells with an IC50 value of $102{,}32\ \mu g/mL$, indicating potential as an anticancer agent, although it is less potent than doxorubicin [10,21]. Furthermore, the extract has been reported to inhibit liver tumor growth and prevent recurrence after cisplatin therapy by suppressing cancer stem cell properties and inhibiting the Wnt/β-catenin signaling pathway [8,22]. This effect is primarily attributed to the inhibition of cancer stem cell (CSC) growth, as evidenced by reduced sphere formation and decreased expression of CSC markers such as CD133, CD44, and EpCAM, which are known to be associated with poor prognosis and early tumor recurrence [19,22]. Polysaccharides isolated from Gynura divaricata also show antitumor effects by inhibiting proliferation and inducing apoptosis in various cancer cell lines [23]. Based on these findings, this plant holds great promise as a herbal-based therapeutic agent for cancer treatment.

CONCLUSIONS

Gynura divaricata is a promising plant with potential as an alternative therapy to complement existing treatments for colorectal cancer. Based on the findings of this study, *Gynura divaricata* demonstrated a significant ability to reduce cancer cell viability. Notably, a concentration of 62.09 µg/mL was able to inhibit 50% of the viability of the cultured cancer cells, highlighting its potential as an effective adjunctive therapeutic agent. Further studies are needed to elucidate the mechanisms underlying its anticancer effects and to assess its safety and efficacy in vivo.

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Author Contributions

FNPR and FNS : Conceptualization, methodology, data curation, original draft preparation. NH and RA : Experimental work, formal analysis, data interpretation. NH : Supervision, review and editing of the manuscript, validation. FNS and NH : Project administration, resources, funding acquisition.

Conflict of Interest

The authors declare no conflict of interest.

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