BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY
Volume 15, Number 1, April 2026 | Pages: 1129-1138 | DOI: 10.14421/biomedich.2026.151.1129-1138
ISSN 2089-6514 (paper)
ISSN 2540-9328 (online)

Evaluation of Antioxidant and Cytotoxicity Activities of *Mimosa pudica* Linn. Leaf Extract Combined with Silver Nanoparticles (AgNPs) Against Human Colorectal Cancer HCT116 Cell

Rahmad Fajral Ilhami[1], Dian Pertiwi[2], Dessy Arisanty[3], Yanti Rahayu[4]
[1]Biomedical Science of Bachelor Program, Faculty of Medicine, Universitas Andalas, Jl. Limau Manis Pauh 26152 Padang, Indonesia.
[2]Clinical Pathology Department, Faculty of Medicine, Universitas Andalas, Jl. Limau Manis Pauh 26152 Padang, Indonesia.
[3]Biochemistry Department, Faculty of Medicine, Universitas Andalas, Jl. Limau Manis Pauh 26152 Padang, Indonesia.
[4]Department of Medical laboratory Technology, Faculty of Health, Universitas Syedza Saintika, Jl. Prof. Dr. Hamka Air Tawar 25132 Padang, Indonesia.

Corresponding author*
ilhamirahmadfajral@gmail.com

Manuscript received: 01 April 2026. Revision accepted: 19 June 2026, Published: 24 June 2026.

Abstract

Colorectal cancer is one of the leading causes of cancer-related morbidity and mortality worldwide, with oxidative stress playing a pivotal role in colorectal carcinogenesis. The limitations of conventional therapies, including systemic toxicity and drug resistance, have driven the exploration of alternative therapeutic agents derived from natural products. *Mimosa pudica* Linn. leaves are known to contain bioactive compounds with antioxidant and anticancer properties; however, their clinical application is limited by poor biopharmaceutical characteristics. The combination of *Mimosa pudica* leaf extract with silver nanoparticles (AgNPs) is expected to enhance its biological efficacy through nanotechnology-based drug delivery systems. This study was conducted as an in vitro experimental investigation. *Mimosa pudica* Linn. leaf extract was prepared using an extraction method and subsequently combined with silver nanoparticles. Antioxidant activity was evaluated using the DPPH assay, with ascorbic acid serving as a positive control. Cytotoxic activity against the human colorectal cancer cell line HCT116 was assessed using the MTT assay to determine cell viability and IC50 values. Statistical analyses were performed to evaluate differences among treatment groups. The results demonstrated that *Mimosa pudica* Linn. leaf extract exhibited significant antioxidant activity, although it was lower than that of ascorbic acid. In cytotoxicity assays, the leaf extract reduced HCT116 cell viability in a dose-dependent manner. Notably, the combination of *Mimosa pudica* leaf extract with silver nanoparticles produced a more pronounced cytotoxic effect compared to the extract alone, as indicated by a greater reduction in cell viability and lower IC50 values. In conclusion, *Mimosa pudica* Linn. leaf extract possesses antioxidant and cytotoxic activities against HCT116 colorectal cancer cells. Its combination with silver nanoparticles enhances cytotoxic potency, highlighting its potential as a nanomedicine-based anticancer candidate for colorectal cancer therapy.

Keywords: Antioxidant; Cytotoxicity; Colorectal Cancer; *Mimosa pudica* Linn.; Silver Nanoparticle.

INTRODUCTION

Colorectal cancer (CRC) remains a major global health burden and represents one of the leading causes of cancer-related morbidity and mortality worldwide. According to recent epidemiological data, CRC ranks as the third most commonly diagnosed malignancy and the second leading cause of cancer-related deaths globally (Kratzer et al., 2023; Siegel et al., 2023). The Global Cancer Observatory (GLOBOCAN) reported in 2022 that CRC accounted for approximately 9.6% of all new cancer cases and 9.3% of cancer-related deaths worldwide. In Indonesia, 35,676 new CRC cases (8.7%) and 19,255 deaths (7.9%) were recorded, highlighting the substantial national disease burden (Bray et al., 2024).

CRC is a multifactorial disease arising from complex interactions between genetic predisposition, lifestyle factors, medical conditions, and environmental exposures (Anbari and Ghanadi, 2025; Li et al., 2024). Among the various pathogenic mechanisms involved, oxidative stress plays a pivotal role in CRC development and progression. Oxidative stress occurs when the generation of reactive oxygen species (ROS) exceeds the neutralizing capacity of endogenous antioxidant systems (Catalano et al., 2025). Excessive ROS accumulation induces oxidative damage to essential cellular biomolecules, including DNA, proteins, and lipids, leading to genomic instability, dysregulation of cellular homeostasis, and activation of aberrant signaling pathways that promote carcinogenesis (Bardelčíková et al., 2023; Liu et al., 2017).

Current therapeutic strategies for CRC primarily involve surgical intervention, chemotherapy, and radiotherapy. Despite significant advances in treatment modalities, their clinical efficacy remains limited by

substantial adverse effects, systemic toxicity, and the development of drug resistance (Fadlallah et al., 2024). Standard chemotherapeutic agents, such as 5-fluorouracil (5-FU) and oxaliplatin (OXP), are associated with severe side effects, including gastrointestinal toxicity, peripheral neuropathy, and bone marrow suppression (Han et al., 2024). Furthermore, chemoresistance represents a major obstacle in CRC management, contributing to disease recurrence and therapeutic failure (Azwar et al., 2021; Gmeiner and Okechukwu, 2023). Recent evidence has demonstrated that elevated expression of Colony Stimulating Factor 2 (CSF2) is significantly associated with chemoresistance in CRC cells, particularly in 5-FU–resistant HCT-8 cells, indicating its potential role as a molecular determinant of treatment failure (Zhou et al., 2025).

These limitations have stimulated increasing interest in the exploration of alternative therapeutic agents that are more selective, safer, and less toxic to normal tissues. In this context, bioactive compounds derived from medicinal plants have emerged as promising candidates due to their relatively low toxicity and multitargeted anticancer properties (Khan et al., 2021).

Mimosa pudica Linn., commonly known as the sensitive plant, has long been utilized in traditional medicine systems. Phytochemical analyses have revealed that *M. pudica* especially leave contains a wide range of bioactive constituents, including alkaloids, flavonoids, tannins, and phenolic compounds (Rizwan et al., 2022). These secondary metabolites exhibit diverse pharmacological activities, particularly antioxidant and anticancer effects. Flavonoids and phenolic compounds are well recognized for their ability to scavenge free radicals, suppress oxidative stress, and inhibit carcinogenic signaling pathways (Zahra et al., 2024). Several preclinical studies have demonstrated that *M. pudica* extracts exert significant and selective cytotoxic effects against various cancer cell lines, mainly through the induction of apoptosis (John et al., 2020; Kumar et al., 2023; Parmar Felisa et al., 2015).

Despite their promising bioactivity, plant-derived extracts often face critical limitations in clinical applications, including poor chemical stability, low aqueous solubility, and limited bioavailability (Muhammad et al., 2016). Previous investigations have indicated that more than 40% of phytochemicals in *M. pudica* leaf, including major bioactive compounds such as quercetin and myricetin, exhibit poor solubility, resulting in restricted intestinal absorption and reduced systemic circulation (Li et al., 2025). These pharmacokinetic constraints significantly compromise their therapeutic efficacy.

Nanotechnology, particularly through green synthesis approaches, has emerged as an innovative strategy to overcome these limitations. Silver nanoparticles (AgNPs) synthesized using plant extracts offer enhanced biocompatibility and functionalization. AgNPs possess unique physicochemical properties that facilitate their penetration into tumor tissues via the enhanced permeability and retention (EPR) effect, thereby promoting preferential drug accumulation at tumor sites and reducing systemic toxicity (Asif et al., 2022; Eker et al., 2025). In green synthesis processes, *M. pudica* leaf extract functions simultaneously as a reducing agent for silver ions (Ag^+) and as a stabilizing or capping agent, resulting in stable and biofunctional nanoparticles.

The integration of *M. pudica* leaf extracts with AgNPs (MP+AgNPs) is expected to generate synergistic therapeutic effects, wherein AgNPs serve as efficient drug delivery carriers, while plant-derived bioactive compounds contribute intrinsic antioxidant and anticancer activities. This synergism may lead to enhanced therapeutic efficacy compared with the individual components alone (Takáč et al., 2023). Although numerous studies have reported the anticancer potential of *M. pudica* leaf extracts and AgNPs independently, systematic investigations focusing on their combined application as nanotherapeutic agents for CRC remain limited (Muhammad et al., 2016). This gap in knowledge represents a critical research opportunity, particularly in the context of sustainable and green nanomedicine development.

In vitro models play a fundamental role in evaluating novel anticancer agents. Several CRC cell lines, including HT29, Caco-2, SW480/SW620, DLD-1, LoVo, and HCT116, are widely used in preclinical research. Among these, HCT116 cells are considered highly representative of human CRC due to their well-characterized genetic and phenotypic profiles (Liu et al., 2023). Derived from colorectal epithelial carcinoma, HCT116 cells exhibit sensitivity to chemotherapeutic agents such as 5-FU and are frequently employed in studies of drug screening, gene therapy, and nanomedicine-based interventions (Salerno et al., 2024). Therefore, this study aimed to evaluate the in vitro antioxidant and cytotoxic activities of *m.pudica* Linn. leaf extract combined with silver nanoparticles against human colorectal cancer HCT116 cells. By integrating phytotherapy and green nanotechnology, this research seeks to provide novel insights into the development of effective, safe, and sustainable nanotherapeutic strategies for colorectal cancer management.

MATERIALS AND METHODS

Materials

This study utilized analytical-grade chemicals and certified biological materials that met standard research quality requirements. Fresh *M.pudica* leaves were carefully selected and authenticated prior to use. Ethanol (96%), methanol (100%), silver nitrate ($AgNO_3$) powder, ascorbic acid, DPPH reagen (2,2-diphenyl-1-picrylhydrazyl), phosphate-buffered saline (PBS),

trypsin–EDTA (0.25%), fetal bovine serum (FBS), penicillin–streptomycin, dimethyl sulfoxide (DMSO), and MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were purchased from Merck (Germany). The human colorectal carcinoma cell line HCT116 (ATCC® CCL-247™) was obtained from the American Type Culture Collection (ATCC, USA). Roswell Park Memorial Institute (RPMI)-1640 medium was used as the basal culture medium.

Preparation of *M.pudica* Leaf Samples

Fresh *M.pudica* leaves were selected based on physical quality, including green coloration, absence of mechanical damage, and lack of visible contamination. The samples were washed thoroughly under running tap water to remove surface impurities and subsequently air-dried in a shaded, well-ventilated environment to prevent degradation of bioactive compounds. The dried plant material was then pulverized into fine powder using a mechanical grinder and stored in airtight containers at room temperature until extraction.

Preparation of *M.pudica* Leaf Extract

Extraction was performed using the maceration method. Briefly, 500 g of dried leaf powder was immersed in 1 L of 96% ethanol and incubated at room temperature for 2–4 days with intermittent agitation. After maceration, the mixture was filtered using Whatman filter paper to remove solid residues. The resulting filtrate was concentrated under reduced pressure using a rotary evaporator at temperatures below 40°C to obtain a viscous crude extract. The concentrated extract was subsequently subjected to phytochemical analysis. The extraction and phytochemical characterization procedures were conducted in accordance with previously established protocols.

Green Synthesis of Silver Nanoparticles Using *M.pudica* Leaf Extract

A stock solution of silver nitrate was prepared by dissolving 8.4 g of $AgNO_3$ in 1 L of distilled water. Subsequently, 500 mL of $AgNO_3$ solution was mixed with 250 mL of *M. pudica* leaf extract in a glass beaker. The mixture was covered with aluminum foil to minimize light exposure due to the photosensitivity of silver ions. The reaction mixture was heated in a water bath at 60°C fFor 60 min. Successful synthesis of silver nanoparticles (AgNPs) was indicated by a visible color change from pale yellow to dark brown, suggesting nanoparticle formation. The synthesized nanoparticles were purified by centrifugation at 3,500 rpm for 20 min. The supernatant was discarded, and the pellet was washed with distilled water two to three times to remove unreacted components. The purified nanoparticles were dried in an oven at 60°C and stored in sterile, airtight containers until further use. The synthesis procedure followed previously reported green synthesis protocols.

DPPH Radical Scavenging Assay

The antioxidant activity of *M. pudica* leaf extract was evaluated using the DPPH radical scavenging assay. A DPPH stock solution was prepared by dissolving 2 mg of DPPH powder in 10 mL of 100% methanol. The solution was homogenized, protected from light using aluminum foil, and stored at 4°C. The crude extract (1.5 g) was dissolved in 1.5 mL of methanol and serially diluted to obtain concentrations of 31.25, 62.5, 125, 250, 500, and 1,000 µg/mL, following the method described by Chandra et al. (2020). Ascorbic acid was used as a positive control and prepared at identical concentrations. For each concentration, 100 µL of sample or control solution was added to six replicate wells of a 96-well microplate, followed by the addition of 100 µL of DPPH solution. The plates were incubated for 30 min at 37°C in the dark. Absorbance was measured at 517 nm using an xMark microplate spectrophotometer. Radical scavenging activity was calculated based on the percentage of DPPH inhibition.

Cell Culture of HCT116 Cells

Human colorectal cancer HCT116 cells were used as an in vitro experimental model. Cryopreserved cells were rapidly thawed in a water bath at 37°C for 1–2 min. The cell suspension was transferred into 15 mL centrifuge tubes containing complete growth medium (RPMI-1640 supplemented with 10% FBS and 1% penicillin–streptomycin) and centrifuged at 1,200 rpm for 4 min to remove cryoprotectants. The cell pellet was resuspended in fresh medium and seeded into 25 cm^2 culture flasks. Cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2. The culture medium was replaced every 2–3 days, and cells were subcultured upon reaching approximately 80% confluence.

MTT Assay

Cell viability was assessed using the MTT assay. HCT116 cells at approximately 80% confluence were detached using 0.25% trypsin–EDTA and counted with a hemocytometer. Cells were seeded into 96-well plates at a density of 2×10^4 cells per well in 100 µL of complete medium and incubated overnight to allow cell attachment. The experimental design included untreated cell controls, medium controls, and treatment groups. Cells were treated with *M. pudica* leaf extract (lMP) and *M. pudica*-mediated silver nanoparticles (dPM+AgNPs) at concentrations of 31.25, 62.5, 125, 250, 500, and 1,000 µg/mL. Each treatment was added in 100 µL volume per well and incubated for 24 h. Following treatment, the culture medium was removed, and 100 µL of MTT solution (0.5 mg/mL) was added to each well. The plates were incubated for 4 h at 37°C to allow formazan crystal formation. Subsequently, 100 µL of DMSO was added to dissolve the formazan crystals, and absorbance was measured at 570 nm using an xMark microplate reader.

Cell viability was expressed as a percentage relative to untreated controls.

Statistical Analysis

All experiments were conducted in triplicate, and data were presented as mean ± standard error mean (SEM). Statistical analysis was performed using the independent t-test to compare differences between two experimental groups in antioxidant and cytotoxicity assays. A p-value of less than 0.05 was considered statistically significant.

RESULT AND DISCUSSION

Result

DPPH Radical Scavenging Activity of *M.pudica* Leaf Extract and Ascorbic Acid

Table 1. Percentage of DPPH Inhibition and IC_{50} Values Determined by the DPPH Assay.

Samples	Concentration (µg/mL)	% DPPH Inhibition Mean ± SEM	IC50 (µg/mL)
M.pudica Leaf Ectract	31,25	10,420 ± 0,015	563
	62,5	13,858 ± 0,049	
	125	16,784 ± 0,037	
	250	27,167 ± 0,023	
	500	44,767 ± 0,039	
	1000	82,592 ± 0,010	
Ascorbic Acid	31,25	63,927 ± 0,262	-1,874
	62,5	88,097 ± 0,066	
	125	96,654 ± 0,003	
	250	96,586 ± 0,001	
	500	96,887 ± 0,001	
	1000	97,270 ± 0,004	

The antioxidant activity of *M.pudica* Linn. leaf extract and ascorbic acid was evaluated using the DPPH radical scavenging assay. As summarized in Table 5.1, the extract exhibited a marked concentration-dependent increase in DPPH inhibition. At the lowest tested concentration (31.25 µg/mL), the extract showed 10.420% inhibition, which increased substantially to 82.592% at 1,000 µg/mL, indicating enhanced free radical scavenging capacity at higher doses. In contrast, ascorbic acid, used as a positive control, demonstrated rapid and potent antioxidant activity. Even at 31.25 µg/mL, ascorbic acid inhibited 63.927% of DPPH radicals and reached a saturation plateau of approximately 97% from 125 µg/mL onward. These findings confirm the superior antioxidant potency of the standard compound compared with the crude plant extract. Both treatment groups exhibited very low standard error of the mean (SEM) values, ranging from 0.001 to 0.262, indicating high experimental precision and excellent reproducibility of the antioxidant assay.

The half-maximal inhibitory concentration (IC_{50}), representing the concentration required to scavenge 50% of DPPH radicals, was also determined (Table 5.1). The *M. pudica* leaf extract showed an IC_{50} value of 563 µg/mL, suggesting relatively weak antioxidant activity. In comparison, ascorbic acid yielded a negative IC_{50} value (−1.874 µg/mL), reflecting a mathematical artifact resulting from inhibition values exceeding 50% at the lowest tested concentration (31.25 µg/mL). Practically, this indicates that the actual IC_{50} of ascorbic acid was below 31.25 µg/mL.

The concentration-dependent inhibition profiles of both the extract and ascorbic acid are illustrated in Figure 1, further demonstrating the progressive increase in antioxidant activity with rising concentrations.

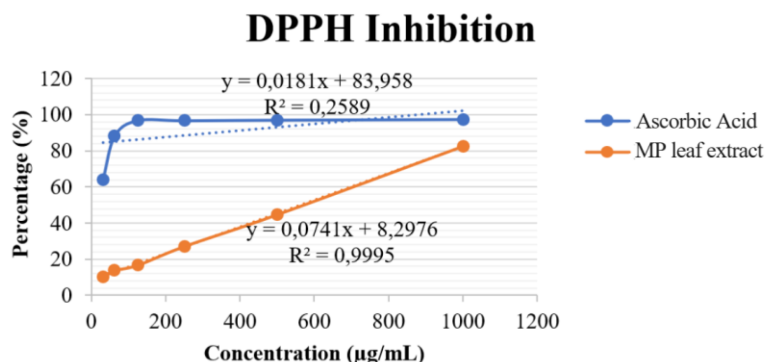

Figure 1. Percentage of DPPH Radical Inhibition Measured by the DPPH Assay in Each Experimental Group.

Viability of HCT116 Cells Treated with *M.pudica* Leaf Extract and Its Combination with Silver Nanoparticles

Morphological alterations of HCT116 colorectal cancer cells following treatment were characterized by the progressive appearance of apoptotic bodies with increasing sample concentrations. The presence of apoptotic bodies indicates metabolically mediated programmed cell death, which corresponds to a reduction in cell viability. Figures 2 and 3 illustrate representative microscopic images of HCT116 cells following 24 h incubation with *M.pudica* leaf extract alone and in combination with silver nanoparticles (AgNPs) at various concentrations.

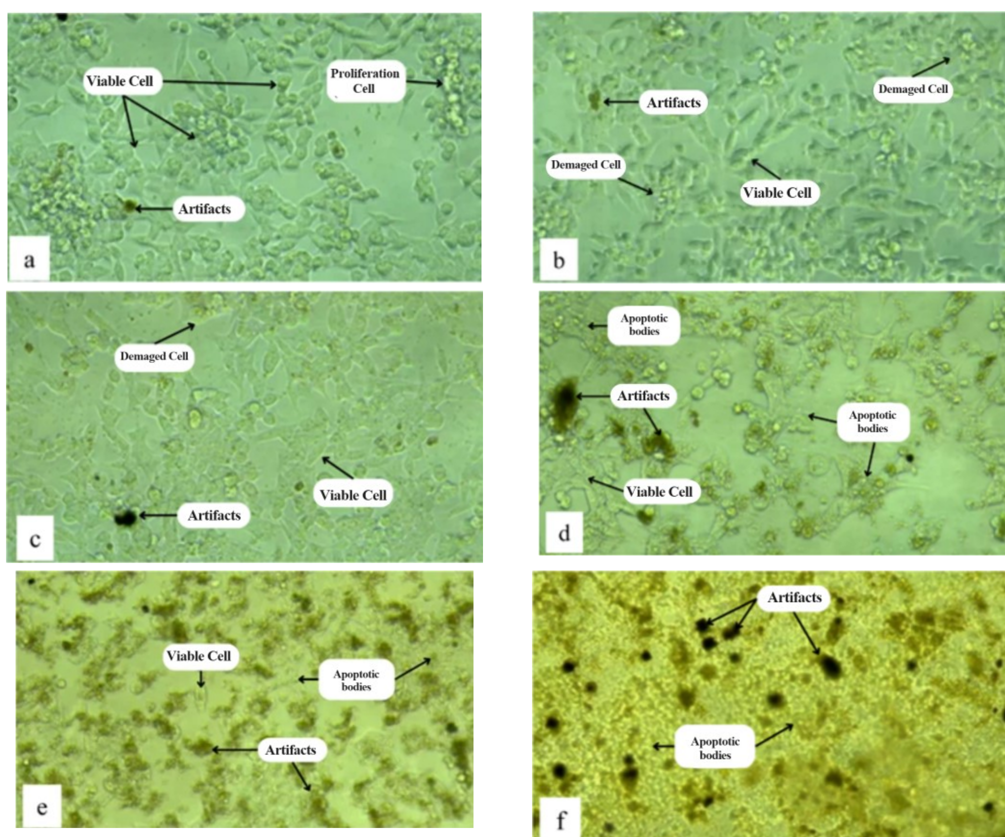

Figure 2. Representative microscopic images (40× magnification) of HCT116 cells after 24 h exposure to *M.pudica* leaf extract at concentrations of (a) 31.25 µg/mL, (b) 62.5 µg/mL, (c) 125 µg/mL, (d) 250 µg/mL, (e) 500 µg/mL, and (f) 1000 µg/mL.

Microscopic observation at 40× magnification revealed that HCT116 cells treated with *M. pudica* leaf extract for 24 h maintained relatively normal polygonal morphology and strong substrate adhesion at low concentrations (31.25–62.5 µg/mL), although initial signs of cellular stress, such as cell rounding, were observed. At intermediate concentrations (125–250 µg/mL), a marked reduction in cell density was evident, accompanied by increased cell rounding, shrinkage, detachment from the culture surface, and the appearance of apoptotic bodies, indicating compromised membrane integrity and initiation of cell death. At higher

concentrations (500–1000 µg/mL), extensive morphological damage was observed, including cell lysis, fragmentation, accumulation of cellular debris, and near-complete loss of monolayer structure, reflecting a substantial decline in cell viability (Figure 2).

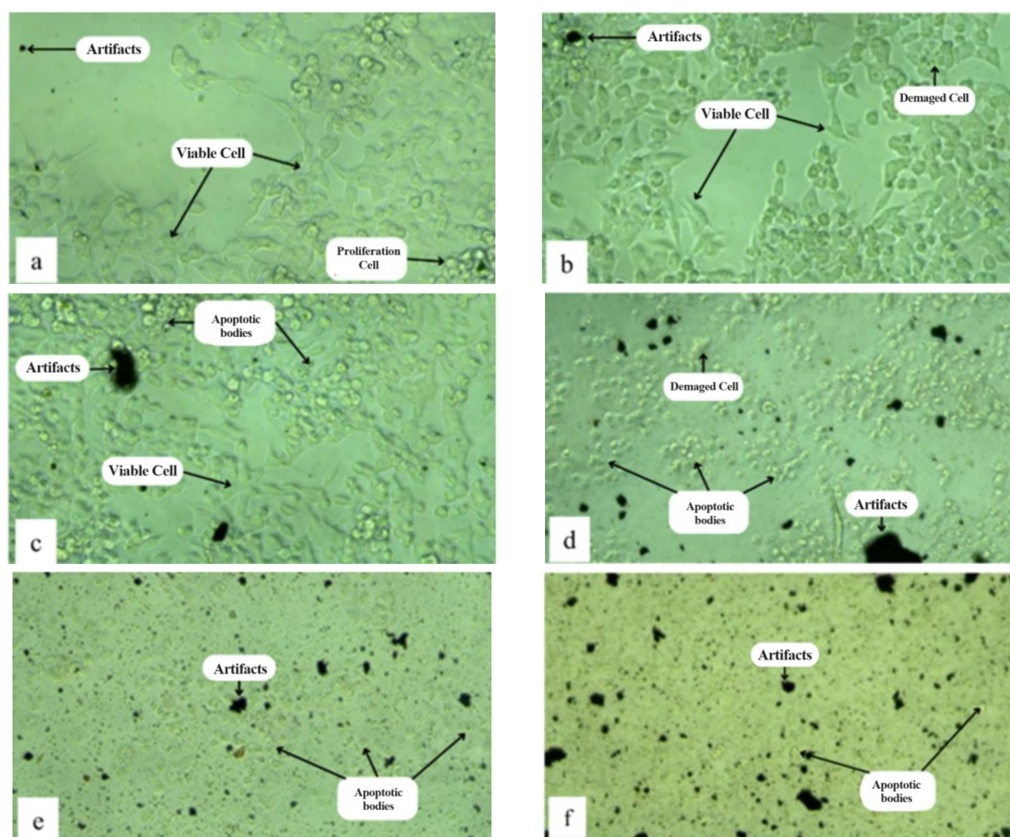

Figure 3. Representative microscopic images (40× magnification) of HCT116 cells after 24 h exposure to the combination of *pudica* leaf extract and silver nanoparticles at concentrations of (a) 31.25 µg/mL, (b) 62.5 µg/mL, (c) 125 µg/mL, (d) 250 µg/mL, (e) 500 µg/mL, and (f) 1000 µg/mL.

As shown in Figure 3, treatment with the combination of *M. pudica* leaf extract and AgNPs induced more rapid and severe morphological alterations compared with the extract alone, in a concentration-dependent manner. Even at low concentrations (31.25–62.5 µg/mL), a noticeable decrease in cell density and increased loss of cell adhesion were observed, suggesting early cytotoxic effects. At moderate concentrations (125–250 µg/mL), pronounced morphological deterioration occurred, characterized by cell fragmentation, cytoplasmic shrinkage, disruption of the monolayer structure, and abundant apoptotic bodies. At the highest concentrations (500–1000 µg/mL), extensive cellular destruction was evident, with dominant cell lysis, fragmentation, and accumulation of apoptotic debris, indicating a severe reduction in cell viability.

Table 2. Percentage of cell viability and IC_{50} values of *M.pudica* leaf extract and its combination with silver nanoparticles determined by the MTT assay.

Samples	Concentration (µg/mL)	% Cell Viability Mean ± SEM	IC50 (µg/mL)
M.pudica Leaf Ectract	31,25	90,308 ± 0,020	504,962
	62,5	80,919 ± 0,027	
	125	74,961 ± 0,035	
	250	64,511 ± 0,064	
	500	52,104 ± 0,028	
	1000	34,106 ± 0,024	
M.pudica Leaf Ectract + Silver Nanoparticle	31,25	87,664 ± 0,034	164,552
	62,5	78,078 ± 0,047	
	125	65,457 ± 0,040	
	250	37,511 ± 0,009	
	500	15,572 ± 0,041	
	1000	4,549 ± 0,013	

Results of the MTT assay are presented as percentage cell viability, reflecting cellular metabolic activity rather than direct cell counts, as indicated by the intensity of purple formazan crystals formed through MTT reduction by viable cells. Both samples exhibited a clear dose-dependent cytotoxic response, with increasing concentrations leading to progressively lower cell viability. Treatment with *M. pudica* leaf extract alone resulted in high cell viability at the lowest concentration (90.31% at 31.25 µg/mL), which gradually decreased to 34.11% at 1000 µg/mL. In contrast, the combination treatment produced a markedly stronger cytotoxic effect, reducing cell viability from 87.66% at 31.25 µg/mL to only 4.55% at 1000 µg/mL (Table 2).

IC_{50} analysis further confirmed the enhanced cytotoxic potency of the combination treatment. The IC_{50} value of the *M. pudica* leaf extract alone was 504.96 µg/mL, indicating weak cytotoxic activity against HCT116 cells. Conversely, the extract–AgNPs combination yielded a substantially lower IC_{50} value of 164.55 µg/mL, demonstrating approximately a threefold increase in anticancer efficacy (Table 2). These findings suggest that silver nanoparticle incorporation significantly enhances the cytotoxic potential of *M. pudica* leaf extract against colorectal cancer cells. The decrease in cell viability percentage can be visually observed in the graph presented on Figure 4.

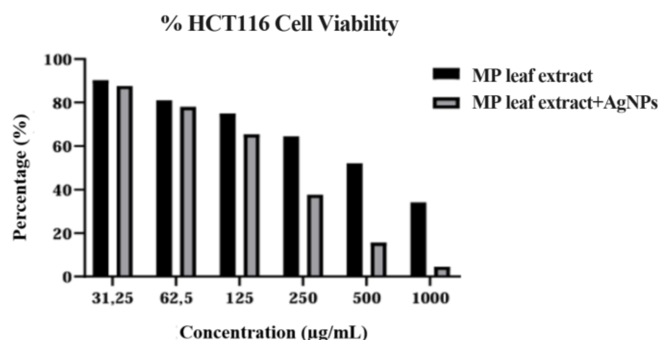

Figure 4. Percentage viability of HCT116 colorectal cancer cells following treatment with *M.pudica* leaf extract and its combination with silver nanoparticles after 24 hours of incubation.

Discussion

Antioxidant Activity of *M.pudica* Leaf Extract and Ascorbic Acid

This study evaluated the antioxidant activity of *M.pudica* Linn. leaf extract using the DPPH radical scavenging assay. The results demonstrated that both the ethanol extract and ascorbic acid exhibited concentration-dependent inhibition of DPPH radicals, indicating dose-responsive antioxidant activity. The percentage inhibition of the *M. pudica* leaf extract increased from 10.42% at 31.25 µg/mL to 82.59% at 1,000 µg/mL, reflecting its ability to neutralize free radicals at higher concentrations.

Ascorbic acid was employed as a positive control to validate the reliability of the assay, owing to its well-established antioxidant properties and rapid hydrogen-donating kinetics (Wulan et al., 2019). In the present study, ascorbic acid showed substantial inhibitory activity even at the lowest tested concentration, achieving 63.93% inhibition at 31.25 µg/mL and reaching a plateau of approximately 96–97% from 125 µg/mL onward. These findings confirm the superior antioxidant potency of pure compounds compared with crude plant extracts.

Statistical analysis revealed a significant difference ($p = 0.006$) between the antioxidant activities of the *M. pudica* leaf extract and ascorbic acid, indicating that the crude extract possesses lower scavenging efficiency. This discrepancy can be attributed to the heterogeneous nature of plant extracts, which contain complex mixtures of bioactive and inactive constituents that may dilute antioxidant effectiveness (Sari and Ernanda, 2021).

The IC_{50} value of the *M. pudica* leaf extract was determined to be 563 µg/mL, classifying it as a weak antioxidant according to Blois' classification. In contrast, ascorbic acid exhibited an apparent negative IC_{50} value (−1.874 µg/mL), resulting from inhibition levels exceeding 50% at the lowest tested concentration, thereby indicating an actual IC_{50} below 31.25 µg/mL. These findings further emphasize the markedly stronger antioxidant capacity of the reference compound.

The present results are consistent with previous studies. Damayanti et al. (2025) reported a similar IC_{50} value of 575.8 µg/mL for ethanol extracts of *M. pudica* leaves, categorizing them as weak antioxidants. Conversely, Rai and Rai (2024) observed strong antioxidant activity in ethyl acetate extracts, with an IC_{50} of 46.58 µg/mL, while Mandal et al. (2022) reported moderate activity (158.95 ± 1.12 µg/mL). These variations may be attributed to differences in environmental conditions, harvesting time, geographical distribution, and extraction solvents, which collectively influence phytochemical composition. Solvent polarity plays a critical role in extracting antioxidant compounds, with ethyl acetate and methanol generally yielding higher phenolic content than ethanol or aqueous solvents (Mandal et al., 2022).

Overall, the antioxidant activity observed in this study indicates the presence of secondary metabolites capable of donating electrons or hydrogen atoms to stabilize free radicals. Although relatively weak, this activity supports the potential of *M. pudica* leaf extract as a natural antioxidant source that may contribute to complementary therapeutic strategies, particularly in diseases associated with oxidative stress, such as colorectal cancer.

Cytotoxic Activity of *M.pudica* Leaf Extract Against HCT116 Cells

The cytotoxic potential of the ethanol extract of *M. pudica* leaves against HCT116 colorectal cancer cells was assessed using the MTT assay. The results demonstrated a concentration-dependent reduction in cell viability, indicating dose-responsive cytotoxicity. At the highest concentration (1,000 µg/mL), cell viability

decreased to 34.11%, whereas at the lowest concentration (31.25 µg/mL), viability remained relatively high (90.31%).

Microscopic observations corroborated these quantitative findings, revealing progressive morphological alterations with increasing extract concentration. Cellular deformation was evident even at low concentrations, while apoptotic bodies became prominent at 250 µg/mL and increased markedly at higher doses. The presence of apoptotic bodies suggests activation of programmed cell death pathways, leading to irreversible loss of cellular metabolic function.

Regression analysis yielded an IC_{50} value of 504.962 µg/mL, classifying the extract as weakly cytotoxic based on established criteria (Nurdiani et al., 2023). This finding aligns with previous reports indicating that crude plant extracts generally require higher concentrations to induce cancer cell death compared with purified compounds.

Limited studies have investigated the cytotoxic effects of *M. pudica* specifically on colorectal cancer cells. Chandra et al. (2020) reported strong cytotoxic activity of ethanol extracts against Dalton's Lymphoma Ascites cells, mediated primarily through membrane damage and lipid peroxidation. Similarly, John et al. (2020) demonstrated potent cytotoxic effects of methanolic extracts against breast cancer cell lines, with substantially lower IC_{50} values. These discrepancies likely reflect variations in cell type sensitivity, extraction solvents, and phytochemical profiles.

Despite its weak cytotoxicity as a single agent, *M. pudica* extract remains clinically relevant as a bioactive precursor rather than a stand-alone chemotherapeutic agent. The IC_{50} value obtained in this study serves as an important baseline, supporting the hypothesis that nanotechnology-based formulations may enhance the intrinsic anticancer potential of this plant extract.

Cytotoxic Activity of *M.pudica*-Mediated Silver Nanoparticles Against HCT116 Cells

The application of green nanotechnology significantly enhanced the cytotoxic efficacy of *M. pudica* extract against HCT116 cells. The integration of the extract as both a reducing and stabilizing agent in silver nanoparticle synthesis resulted in markedly improved antiproliferative activity compared with the crude extract alone.

MTT assay results demonstrated a pronounced, concentration-dependent decline in cell viability following treatment with the extract–AgNPs formulation. Morphological analysis revealed early cellular damage at low concentrations and extensive apoptotic body formation from 125 µg/mL onward. At the highest concentration (1,000 µg/mL), nearly complete cellular destruction was observed, indicating potent anticancer activity. Quantitatively, cell viability decreased from 87.66% at the lowest concentration to only 4.55% at the highest dose. Statistical analysis confirmed the significance of this reduction ($p = 0.035$), indicating that the combination treatment exerted a significantly stronger cytotoxic effect than the crude extract. Moreover, the IC_{50} value of the nanoparticle formulation (164.552 µg/mL) was approximately three-fold lower than that of the extract alone, demonstrating substantial enhancement of anticancer potency.

Although direct studies on *M. pudica*-based AgNPs remain limited, similar findings have been reported using other plant extracts. Almukaynizi et al. (2022) demonstrated that silver nanoparticles synthesized from Adansonia digitata significantly improved cytotoxicity against HCT116 and SW480 cells. Furthermore, Shukla and Iravani (2024) highlighted that the nanoscale size and large surface area of AgNPs facilitate efficient cellular interactions and uptake. Gomes et al. (2023) further suggested that phytochemical coatings on nanoparticles promote synergistic interactions between silver ions and plant metabolites, thereby enhancing anticancer activity.The enhanced cytotoxicity observed in this study may be attributed to improved cellular internalization, sustained intracellular silver ion release, and synergistic oxidative and apoptotic mechanisms. Additionally, nanocarrier systems have been shown to increase therapeutic indices, enabling effective tumor cell eradication at lower doses (Okafor et al., 2013).

Collectively, these findings validate the effectiveness of green-synthesized *M. pudica* leaf-mediated AgNPs as a promising nanotherapeutic platform for colorectal cancer. This approach not only enhances the intrinsic bioactivity of plant-derived compounds but also supports the development of environmentally sustainable and potentially safer anticancer agents.

CONCLUSION

The ethanol extract of *M.pudica* Linn. leaves exhibited measurable antioxidant activity with the ability to neutralize free radicals; however, its potency remained relatively weak when compared with pure ascorbic acid as a reference standard. In contrast, the combination of *M. pudica* leaf extract with silver nanoparticles demonstrated significantly enhanced cytotoxic activity, effectively reducing cell viability and suppressing the proliferation of HCT116 colorectal cancer cells. These findings indicate that nanoparticle-assisted delivery improves the anticancer efficacy of the extract compared with its crude form.

Acknowledgements: The authors would like to express their sincere gratitude to the Faculty of Medicine and the affiliated research laboratories for providing the facilities and technical support necessary for the completion of this study. Appreciation is also extended to all laboratory

staff and colleagues who contributed to sample preparation, data collection, and analysis. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The authors declare that there are no financial, personal, or institutional conflicts of interest that could have influenced the outcomes of this work.

Authors' Contributions: Dian Pertiwi and Dessy Arisanty designed the study. Rahmad Fajral Ilhami carried out the laboratory work, analyzed the data and wrote the manuscript. All authors read and approved the final version of the manuscript.

Competing Interests: The authors declare that there are no competing interests.

REFERENCES

Anbari, K., & Ghanadi, K. (2025). Colorectal Cancer: Risk Factors, Novel Approaches in Molecular Screening and Treatment. *International Journal of Molecular and Cellular Medicine*, *14*(1), 576–605. https://doi.org/10.22088/IJMCM.BUMS.14.1.576

Asif, M., Yasmin, R., Asif, R., Ambreen, A., Mustafa, M., & Umbreen, S. (2022). Green Synthesis of Silver Nanoparticles (AgNPs), Structural Characterization, and their Antibacterial Potential. *Dose-Response*, *20*(2). https://doi.org/10.1177/15593258221088709

Azwar, S., Seow, H. F., Abdullah, M., Faisal Jabar, M., & Mohtarrudin, N. (2021). Recent Updates on Mechanisms of Resistance to 5-Fluorouracil and Reversal Strategies in Colon Cancer Treatment. *Biology*, *10*(9), 854. https://doi.org/10.3390/biology10090854

Bardelčíková, A., Šoltys, J., & Mojžiš, J. (2023). Oxidative Stress, Inflammation and Colorectal Cancer: An Overview. *Antioxidants*, *12*(4), 901. https://doi.org/10.3390/antiox12040901

Bray, F., Laversanne, M., Sung, H., Ferlay, J., Siegel, R. L., Soerjomataram, I., & Jemal, A. (2024). Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, *74*(3), 229–263. https://doi.org/10.3322/caac.21834

Catalano, T., Selvaggi, F., Cotellese, R., & Aceto, G. M. (2025). The Role of Reactive Oxygen Species in Colorectal Cancer Initiation and Progression: Perspectives on Theranostic Approaches. *Cancers*, *17*(5), 752. https://doi.org/10.3390/cancers17050752

Damayanti, N. M. D., Sudira, I. W., Bebas, W., Luh Made Sudimartini, & Raharjo, Y. Y. C. Y. A. (2025). Antioxidant Potential Test Of Ethanol Extract Of *Mimosa Pudica* Linn As Herbal Medicine Ingredients. *Buletin Veteriner Udayana*, 549–555. https://doi.org/10.24843/bulvet.2025.v17.i02.p32

Eker, F., Akdaşçi, E., Duman, H., Bechelany, M., & Karav, S. (2025). Green Synthesis of Silver Nanoparticles Using Plant Extracts: A Comprehensive Review of Physicochemical Properties and Multifunctional Applications. *International Journal of Molecular Sciences*, *26*(13), 6222. https://doi.org/10.3390/ijms26136222

Fadlallah, H., El Masri, J., Fakhereddine, H., Youssef, J., Chemaly, C., Doughan, S., & Abou-Kheir, W. (2024). Colorectal cancer: Recent advances in management and treatment. *World Journal of Clinical Oncology*, *15*(9), 1136–1156. https://doi.org/10.5306/wjco.v15.i9.1136

Gmeiner, W. H., & Okechukwu, C. C. (2023). Review of 5-FU resistance mechanisms in colorectal cancer: clinical significance of attenuated on-target effects. *Cancer Drug Resistance*, *6*(2), 257–272. https://doi.org/10.20517/cdr.2022.136

Han, C. J., Ning, X., Burd, C. E., Spakowicz, D. J., Tounkara, F., Kalady, M. F., Noonan, A. M., McCabe, S., & Von Ah, D. (2024). Chemotoxicity and Associated Risk Factors in Colorectal Cancer: A Systematic Review and Meta-Analysis. *Cancers*, *16*(14), 2597. https://doi.org/10.3390/cancers16142597

John, R., Kariyil, B., A. Usha, P., Surya, S., Anu, G., John, P., Sujith, S., & Zarina, A. (2020). In vitro antitumor potential of methanol extract of *Mimosa pudica* in human breast cancer cell lines. *Pharmacognosy Magazine*, *16*(70), 396. https://doi.org/10.4103/pm.pm_527_19

Khan, A. Q., Rashid, K., AlAmodi, A. A., Agha, M. V., Akhtar, S., Hakeem, I., Raza, S. S., & Uddin, S. (2021). Reactive oxygen species (ROS) in cancer pathogenesis and therapy: An update on the role of ROS in anticancer action of benzophenanthridine alkaloids. *Biomedicine & Pharmacotherapy*, *143*, 112142. https://doi.org/10.1016/j.biopha.2021.112142

Kratzer, T. B., Jemal, A., Miller, K. D., Nash, S., Wiggins, C., Redwood, D., Smith, R., & Siegel, R. L. (2023). Cancer statistics for <scp>A</scp> merican <scp>I</scp> ndian and <scp>A</scp> laska <scp>N</scp> ative individuals, 2022: Including increasing disparities in early onset colorectal cancer. *CA: A Cancer Journal for Clinicians*, *73*(2), 120–146. https://doi.org/10.3322/caac.21757

Kumar, A., Gautam, V., Sandhu, A., Rawat, K., Sharma, A., & Saha, L. (2023). Current and emerging therapeutic approaches for colorectal cancer: A comprehensive review. *World Journal of Gastrointestinal Surgery*, *15*(4), 495–519. https://doi.org/10.4240/wjgs.v15.i4.495

Li, B., Zou, R., Su, F., Zhang, W., Liu, M., Cui, N., Bai, H., Zeng, Y., Sun, Y., Wang, Q., & Kuang, H. (2025). Targeted isolation and antipyretic mechanisms of 5,7,4′-trimethoxyflavone and p-hydroxybenzoic acid from *Mimosa pudica* root via cell membrane-coated magnetic carbon spheres integrated with thermal shift assay. *Bioorganic Chemistry*, *163*, 108706. https://doi.org/10.1016/j.bioorg.2025.108706

Li, Q., Geng, S., Luo, H., Wang, W., Mo, Y.-Q., Luo, Q., Wang, L., Song, G.-B., Sheng, J.-P., & Xu, B. (2024). Signaling pathways involved in colorectal cancer: pathogenesis and targeted therapy. *Signal Transduction and Targeted Therapy*, *9*(1), 266. https://doi.org/10.1038/s41392-024-01953-7

Liu, H., Liu, X., Zhang, C., Zhu, H., Xu, Q., Bu, Y., & Lei, Y. (2017). Redox Imbalance in the Development of Colorectal Cancer. *Journal of Cancer*, *8*(9), 1586–1597. https://doi.org/10.7150/jca.18735

Liu, L., Yan, Q., Chen, Z., Wei, X., Li, L., Tang, D., Tan, J., Xu, C., Yu, C., Lai, Y., Fan, M., Tao, L., Shen, W., Li, L., Wu, M., Cheng, H., & Sun, D. (2023). Overview of research progress and application of experimental models of colorectal cancer. *Frontiers in Pharmacology*, *14*. https://doi.org/10.3389/fphar.2023.1193213

Mandal, A. K., Pandey, A., Sah, R. K., Baral, A., & Sah, P. (2022). In Vitro Antioxidant and Antimicrobial Potency of

Mimosa pudica of Nepalese Terai Region: Insight into L-Mimosine as an Antibacterial Agent. *Evidence-Based Complementary and Alternative Medicine*, *2022*, 1–11. https://doi.org/10.1155/2022/6790314

Muhammad, G., Hussain, M. A., Jantan, I., & Bukhari, S. N. A. (2016). *Mimosa pudica* L., a High-Value Medicinal Plant as a Source of Bioactives for Pharmaceuticals. *Comprehensive Reviews in Food Science and Food Safety*, *15*(2), 303–315. https://doi.org/10.1111/1541-4337.12184

Nurdiani, E., Masriani, M., Rasmawan, R., Muharini, R., & Sartika, R. P. (2023). Sitotoksisitas dan Selektivitas Fraksi Kayu Batang Simpur Air (Dillenia suffruticosa (Griff.) Martelli) Terhadap Sel Kanker Payudara. *Al-Kauniyah: Jurnal Biologi*, *17*(1), 190–200. https://doi.org/10.15408/kauniyah.v17i1.31299

Okafor, F., Janen, A., Kukhtareva, T., Edwards, V., & Curley, M. (2013). Green Synthesis of Silver Nanoparticles, Their Characterization, Application and Antibacterial Activity. *International Journal of Environmental Research and Public Health*, *10*(10), 5221–5238. https://doi.org/10.3390/ijerph10105221

Parmar Felisa, Kushawaha Nisha, Highland Hyancinth, & George Linz-buoy. (2015). In Vitro Antioxidant And Anticancer Activity Of *Mimosa Pudica* Linn Extract And L-Mimosine On Lymphoma Daudi Cells. *International Journal of Pharmacy and Pharmaceutical Sciences*, *7*(12), 100–104.

Rai, B., & Rai, J. P. (2024). Phytochemical Screening of *Mimosa pudica* aerial part and evaluation of itspotent antiulcereffect in terms of phenolic and flavonoid components. *Frontiers in Health Informatics*, *13*(3), 11053–11066.

Rizwan, K., Majeed, I., Bilal, M., Rasheed, T., Shakeel, A., & Iqbal, S. (2022). Phytochemistry and Diverse Pharmacology of Genus Mimosa: A Review. *Biomolecules*, *12*(1), 83. https://doi.org/10.3390/biom12010083

Salerno, L., Notaro, A., Consoli, V., Affranchi, F., Pittalà, V., Sorrenti, V., Vanella, L., Giuliano, M., & Intagliata, S. (2024). Evaluation of the anticancer effects exerted by 5-fluorouracil and heme oxygenase-1 inhibitor hybrids in HTC116 colorectal cancer cells. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *39*(1). https://doi.org/10.1080/14756366.2024.2337191

Sari, D. E. M., & Ernanda, T. H. (2021). Uji Aktivitas Antioksidan Krim Ekstrak Daun Mengkudu (Morinda Citrifolia L.) Berbasis Vanishing Cream. *Jurnal Ilmiah JOPHUS: Journal Of Pharmacy UMUS*, *3*(01), 10–18. https://doi.org/10.46772/jophus.v3i01.519

Siegel, R. L., Miller, K. D., Wagle, N. S., & Jemal, A. (2023). Cancer statistics, 2023. *CA: A Cancer Journal for Clinicians*, *73*(1), 17–48. https://doi.org/10.3322/caac.21763

Sri Rashmy Madathil, Poornima Kannappan, Devaki Kanakasabapathy, Sincy Varghese, & Perumalsamy Muneeswari. (2020). Phytochemical and chromatographic characterization of Mimosa diplotricha Wright ex. Sauvalle ethanolic leaf extract and assessment of antioxidant potential and anti-proliferative effect on colorectal cancer (HCT-116) cell lines. *International Journal of Research in Pharmaceutical Sciences*, *11*(3), 4378–4387. https://doi.org/10.26452/ijrps.v11i3.2656

Takáč, P., Michalková, R., Čižmáriková, M., Bedlovičová, Z., Balážová, Ľ., & Takáčová, G. (2023). The Role of Silver Nanoparticles in the Diagnosis and Treatment of Cancer: Are There Any Perspectives for the Future? *Life*, *13*(2), 466. https://doi.org/10.3390/life13020466

Wulan, W., Yudistira, A., & Rotinsulu, H. (2019). Uji Aktivitas Antioksidan Dari Ekstrak Etanol Daun *Mimosa Pudica* Linn. Menggunakan Metode Dpph. *Pharmacon*, *8*(1), 106. https://doi.org/10.35799/pha.8.2019.29243

Zahra, M., Abrahamse, H., & George, B. P. (2024). Flavonoids: Antioxidant Powerhouses and Their Role in Nanomedicine. *Antioxidants*, *13*(8), 922. https://doi.org/10.3390/antiox13080922

Zhou, H., Gao, Z., Wu, X., Wang, Y., & Zhang, L. (2025). CSF2 promotes chemoresistance in colorectal cancer by regulating Notch pathway. *Discover Oncology*, *16*(1), 495. https://doi.org/10.1007/s12672-025-02285-w