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Phyto therapeutic potential of *Andrographis paniculata* and *Catharanthus roseus* extract against colorectal cancer HCT-116 cell line

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ABSTRACT

Colorectal cancer (CRC) is the third most common cancer globally and the second leading cause of cancer-related mortality, with over 1.9 million new cases and 935,000 deaths reported in 2020. Despite therapeutic advances, recurrence, drug resistance, and systemic toxicity remain major challenges. Natural products with antioxidant and cytotoxic activity are increasingly investigated as complementary therapies. *Andrographis paniculata* (Sambiloto), rich in andrographolide, exerts anticancer effects by inducing apoptosis, inhibiting migration and invasion, and modulating PI3K/Akt and NF-κB signalling pathway. *Catharanthus roseus* (Tapak Dara) produces vinca alkaloids, including vincristine and vinblastine, which inhibit microtubule polymerization and are widely used in chemotherapy. Combining these extracts may enhance efficacy and reduce toxicity through synergistic interactions. This in vitro study assessed the cytotoxic and synergistic effects of *A. paniculata* extract (APE) and *C. roseus* extract (CRE) on HCT-116 colorectal cancer cells. Extracts were prepared by ethanol maceration, and cytotoxicity was evaluated using the MTT assay at concentrations ranging from 5 to 100 μg/mL. IC_{50} values were calculated using linear regression, and the combination index (CI) was determined at 1, 1/2 and 1/4 IC_{50} to evaluate synergism. APE and CRE exhibited comparable cytotoxicity, with IC_{50} values of 90 μg/mL and 89.5 μg/mL, respectively. The combination treatment revealed synergistic effects (CI < 1) at multiple ratios, particularly at 1/4 IC_{50} (CI = 0.58), demonstrating enhanced cytotoxicity at reduced concentrations. Both APE and CRE demonstrated significant cytotoxic effects against HCT-116 cells. Their combination produced synergistic interactions, suggesting potential as complementary phytotherapeutic agents for CRC with the benefits of dose reduction and minimized toxicity. Further in vivo and mechanistic studies are warranted.

ABSTRAK

Kanker kolorektal (CRC) merupakan kanker ketiga terbanyak di dunia dan penyebab kematian kedua akibat kanker, dengan lebih dari 1,9 juta kasus baru dan 935.000 kematian dilaporkan pada tahun 2020. Meskipun terdapat kemajuan terapi, kekambuhan, resistensi obat, dan toksisitas sistemik masih menjadi hambatan utama. Bahan alam dengan aktivitas antioksidan dan sitotoksik semakin banyak diteliti sebagai terapi komplementer. *Andrographis paniculata (*Sambiloto), kaya akan andrografolida, memberikan efek antikanker dengan menginduksi apoptosis, menghambat migrasi dan invasi, serta memodulasi jalur pensinyalan PI3K/Akt dan NF-κB. *Catharanthus roseus* (Tapak Dara) menghasilkan alkaloid vinca, termasuk vinkristin dan vinblastin, yang menghambat polimerisasi mikrotubulus dan banyak digunakan dalam kemoterapi. Kombinasi ekstrak-ekstrak ini dapat meningkatkan efikasi dan mengurangi toksisitas melalui interaksi sinergis. Penelitian in vitro ini menilai efek sitotoksik dan sinergis ekstrak *A. paniculata* (APE) dan *C. roseus* (CRE) pada sel kanker kolorektal HCT-116. ekstrak disiapkan dengan maserasi etanol, kemudian sitotoksisitas dievaluasi dengan uji MTT pada konsentrasi 5–100 μg/mL. Nilai IC_{50} ditentukan menggunakan regresi linier, sedangkan indeks kombinasi (*Combination Index*/CI) ditentukan pada 1, 0,5, dan 0,25 IC_{50} untuk mengevaluasi sinergisme. APE dan CRE

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menunjukkan sitotoksisitas yang sebanding, dengan nilai IC_{50} masing-masing 90 µg/mL dan 89,5 µg/mL. Perlakuan kombinasi menghasilkan efek sinergis (CI < 1) pada beberapa rasio, terutama pada 0,25 IC_{50} (CI = 0,58), yang menunjukkan peningkatan sitotoksisitas pada konsentrasi rendah. APE dan CRE menunjukkan efek yang signifikan terhadap sel HCT-116. Kombinasi keduanya menghasilkan interaksi sinergis, menunjukkan potensi sebagai agen fitoterapi komplementer pada CRC dengan manfaat pengurangan dosis dan minimalisasi toksisitas. Penelitian in vivo dan mekanisme lebih lanjut diperlukan untuk validasi efikasi dan keamanan.

INTRODUCTION

Colorectal cancer (CRC) represents a major global health challenge and is increasingly recognized as a leading contributor to cancer-related morbidity and mortality. According to the GLOBOCAN 2020 database, CRC accounted for over 1.9 million new cases and approximately 935,000 deaths worldwide, ranking as the third most frequently diagnosed cancer and the second leading cause of cancer mortality.[1] Alarmingly, the global incidence of CRC is projected to rise by nearly 60% within the next two decades, reaching more than 3.2 million new cases and 1.6 million deaths annually by 2040 if current trends persist.[2] The burden is not limited to high-income nations; in low- and middle-income countries, including several in Southeast Asia, CRC incidence has been steadily increasing in parallel with urbanization, lifestyle changes, and aging populations.[3] In Indonesia, for instance, CRC is among the top five most prevalent cancers, with mortality rates continuing to climb despite advances in screening and treatment.[4] Conventional therapies—comprising surgical resection, chemotherapy, radiotherapy, and, in selected cases, targeted agents—have significantly improved survival in early-stage disease; however, their effectiveness remains unsatisfactory in advanced or metastatic CRC. Major limitations include delayed diagnosis, systemic toxicity, recurrence, and the development of chemoresistance, which together underscore the urgent need for novel and more effective therapeutic strategies.[5]

The development and progression of CRC are closely associated with oxidative stress, characterized by an imbalance between the generation of reactive oxygen species (ROS) and the capacity of cellular antioxidant defences. Excessive ROS production contributes to DNA base modifications, strand breaks, and chromosomal instability, which in turn accelerate the accumulation of genetic and epigenetic alterations driving colorectal tumorigenesis.[6] Beyond direct genotoxic effects, ROS act as secondary messengers that activate multiple oncogenic signaling cascades—including MAPK/ERK, PI3K/Akt, and NF-κB pathways—thereby promoting cell proliferation, survival, angiogenesis, and metastasis.[7] Sustained oxidative stress also fosters a pro-inflammatory tumor microenvironment, further enhancing malignant transformation and progression.[8] Importantly, ROS overproduction has been implicated in chemoresistance, as tumor cells exploit redox adaptation mechanisms to survive cytotoxic stress induced by conventional therapies. Elevated antioxidant enzymes in cancer cells, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, may neutralize therapy-induced oxidative damage, thus limiting treatment efficacy.[9] These insights have led to increasing interest in therapeutic strategies that modulate redox balance—either by suppressing ROS to prevent tumor initiation or by exploiting excessive ROS to selectively trigger cancer cell death. Natural compounds with intrinsic antioxidant and pro-apoptotic properties have therefore gained attention as potential adjuncts in CRC management, offering dual benefits of mitigating oxidative damage in normal cells while sensitizing malignant cells to apoptosis or ferroptosis.[10]

Natural products are an important source of anticancer agents, and among them, *Andrographis paniculata* (Sambiloto) has been extensively studied. Its main bioactive compound, andrographolide, exhibits multiple anticancer mechanisms, including the inhibition of cell proliferation, migration, and invasion, as well as the induction of apoptosis.[11,12] Mechanistically, andrographolide interferes with oncogenic signalling such as PI3K/Akt and NF-κB pathways, enhances radiosensitivity, and has recently been shown to trigger ferroptosis in malignant cells.[13,14] Previous studies demonstrated that andrographolide significantly suppressed glycolysis and induced apoptosis in HCT-116 colorectal cancer cells via PI3K-Akt-mTOR inhibition.[15] Another medicinal plant with established anticancer properties is *Catharanthus roseus* (Tapak Dara), which produces indole alkaloids including vincristine and vinblastine. These alkaloids are clinically applied as chemotherapeutic agents due to their ability to inhibit microtubule assembly, leading to mitotic arrest and apoptosis.[16] In addition, crude extracts of *Catharanthus roseus* have demonstrated cytotoxic and antioxidant activities against several cancer cell lines, supporting its traditional use in anticancer therapy.[12]

Andrographis paniculata and *Catharanthus roseus* combination may provide a synergistic therapeutic effect because of their different but complementary modes of action. Such an approach could enhance cytotoxic efficacy while allowing for dose reduction, thereby minimizing systemic side effects commonly associated with chemotherapy. Synergistic combinations of natural compounds have been increasingly explored to overcome drug resistance and target multiple cancer hallmarks simultaneously.[13,17] Therefore, the present study aims to investigate the cytotoxic potential of combined extracts of *Andrographis paniculata* and *Catharanthus roseus* against the human colorectal cancer cell line HCT-116 in vitro. This study is expected to provide preliminary evidence supporting the development of a phytotherapeutic combination as a complementary approach in CRC therapy.

MATERIALS AND METHODS

Study design

This in vitro experimental study was designed to evaluate the cytotoxic and synergistic effects of *Andrographis paniculata* extract (APE) and *Catharanthus roseus* extract (CRE) on HCT116 human colorectal cancer cells. This study was conducted at the Stem Cell and Cancer Research (SCCR) integrated Laboratory, Semarang, Indonesia.

Sample collection and extract preparation

The aerial parts of *Andrographis paniculata* and *Catharanthus roseus* herbs *were* obtained from Gunungpati, Central Java, Indonesia, and stored in a cooler bag to be transported to the laboratory. Subsequently, the plant material was dried using a cabinet dryer at 40 - 50°C.

The water content of dried *Andrographis paniculata* and *Catharanthus roseus* was tested to ensure compliance with Farmakope Herbal Indonesia 2nd Edition standards before undergoing size reduction using a 60-mesh grinder. The *Andrographis paniculata* and *Catharanthus roseus* powder were extracted separately through maceration using the 96% ethanol solvent in 1:10 ratio for 72 hours at room temperature. Upon filtration, the collected filtrate was subsequently evaporated using vacuum rotary evaporator (RV 10 digital V, IKA®, Staufen, Germany) at 40 - 50°C to yield a concentrated extract. The remaining solvent in the viscous extract was evaporated using a water bath. The *Andrographis paniculata* (APE) and *Catharanthus roseus* extract (CRE) was stored at −20 °C until further use.

Cell culture and herbal extract treatments

Colorectal cancer HCT116 cells were acquired from the culture collection of Stem Cell and Cancer Research (SCCR) integrated Laboratory, Semarang, Indonesia. The HCT116 cells were counted using hemacytometer, and seeded according to the laboratory protocol. Then, the cultured cells were inputted into a 96-microwell with 10^4 cells seeding density.

The HCT116 cells were cultured and maintained in McCoy's 5A (modified) Medium (Gibco, Cat. No. 16600082, MA, USA), supplemented with 10% fetal bovine serum (FBS) (Gibco, Cat. No. A5256701, MA, USA) and 1.5% Penicillin-streptomycin antibiotic solutions (Gibco, Cat. No. 15070063, MA, USA) in the CO_2 incubator (37°C) for 24 hours. After the cultured cells reached 80% confluency, they were treated with APE and CRE in serial doses.

APE and CRE were diluted with 10 mL of dimethyl sulfoxide (DMSO) in amounts of 10 mg each. (Merck, Cat. No. 102952, Darmstadt, Germany), and the solution will be sequentially diluted to make 7 varieties extract concentration (5; 10; 20; 40; 60; 80; 100μg/mL) and placed in triplicate. The plates will be incubated again in the CO_2 incubator (37°C) for 24 hours. Cells that were not treated served as the untreated control.

Cytotoxicity assessment using the MTT assay

The MTT assay was utilized to assess cell viability with a minor adjustment. The reagent of MTT assay was made by diluting 5 mg MTT powder (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Sigma-Aldrich, Cat. No. CT01-5, MO, USA) in 10 mL PBS. 1 mL MTT reagent were diluted in 9 mL medium, then 100 μL of the solution was added to the wells. Post 24 h-treatment, the HCT116 cell culture medium was discarded and replaced with new complete medium containing 0.5 mg/mL of MTT and further incubated for 4 hours. After incubation, 100 µL of DMSO was added to each well and incubated again for 15 minutes. Subsequently, the absorbance was determined using Multiskan SkyHigh Microplate Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) at 595 nm. Cell viability percentages were determined by comparing the absorbance values of the treated samples to those of the untreated controls at various concentrations.[1]

$$\text{Cell viability (\%)} = \frac{(\text{Treated Cell Absorbance} - \text{Media Absorbance})}{(\text{Untreated Control Cell Absorbance} - \text{Media Absorbance})} \times 100$$

The IC_{50} value was determined by performing linear regression between the concentration (x) and % cell viability (y), resulting in the equation y = Bx + A. By using the linear regression, the x value at y = 50% was identified as the IC_{50} value, representing the concentration that inhibits 50% of HCT116 cell proliferation. The experimental data for this research were obtained from three independent replication experiments.

Synergistic Effect Evaluation of APE and CRE on HCT116 Cells

The MTT test was used in the first combination study to evaluate the effects of a particular concentration of APE and CRE, as well as their combination, on HCT116 cells. HCT116 cell were plated in a 96-well microplate at 10^4 cells seeding density and incubated at 37°C for 24 hours. Based on the IC50 value from a single cytotoxicity test, the concentration series was made of 1/2, and 1/4 of IC50 for APE and CRE. Subsequently, the cells were exposed to the predetermined combination of APE and CRE for 24 hours. Following the treatment, the cell viability was determined utilizing the aforementioned in vitro cytotoxicity assay. The following equation was used to evaluate the combination synergism based on previous related research.[2]

$$\text{Combination Index (CI)} = \frac{(D)1}{(Dx)1} + \frac{(D)2}{(Dx)2}$$

(D)1 and (D)2 denote the concentrations of the two compounds used in combination to produce the same effect, while (Dx)1 and (Dx)2 represent the concentrations of each compound required to achieve a specific effect (IC_{50} on HCT116 cell growth).

Statistical analysis

Statistical evaluation of the cell viability data from the combination treatment was performed using a one-way ANOVA, followed by Tukey's post hoc test, with SPSS version 24 software.

RESULTS

APE and CRE treatment potentiates increased apoptosis in HCT-116 cells

The single cytotoxicity assay was conducted to determine the cytotoxic potential of *Andrographis paniculata* and *Catharanthus roseus*, which was subsequently used as the basis for evaluating the synergism of both extracts in the combination assay. The antiproliferative activity in the single assay was assessed using the 50% inhibitory concentration (IC_{50}) parameter, defined as the concentration at which 50% growth inhibition occurs in the cell population. A lower IC_{50} value indicates higher cytotoxic potency of a compound against cancer cells. The cytotoxic effect of a compound can be demonstrated through cell viability measurements. The results of the single assay showed that both APE and CRE exhibited cytotoxic activity against HCT-116 colorectal cancer cells (FIGURE 1), with relatively similar IC_{50} values: APE had an IC_{50} of 90 µg/mL, while CRE had an IC_{50} of 89.5 µg/mL.

Combination index analysis of APE and CRE in HCT-116 cells

The Combination Index (CI) values are used to identify antagonistic (CI > 1), additive (CI = 1), or synergistic (CI < 1) effects. From the obtained data, the combination of *APE* and *CRE* demonstrated synergistic effects (CI < 1) at several concentration ratios, namely APE 0.5 with CRE 0.5 (CI = 0.87), APE 0.5 with CRE 0.25 (CI = 0.74), APE 0.25 with CRE 1 (CI = 0.87), and APE 0.25 with CRE 0.25 (CI = 0.58). Specifically, the most synergistic combination was observed at APE 0.25 and CRE 0.25 (CI = 0.58). These findings clearly indicate that synergistic effects (CI < 1) were achieved at concentration ratios of (0.5:0.5), (0.5:0.25), (0.25:1), and (0.25:0.25). The strongest synergism, with the lowest CI value (0.42), was found at the 0.25:0.25 ratio, corresponding to one-quarter of the IC50 values of each extract when tested individually. This suggests that at this ratio, both extracts effectively cooperated to inhibit the growth of HCT-116 cancer cells, even at doses lower than the individual IC50 values. Such synergistic effects are particularly important, as they may allow for the use of lower doses of each component, potentially reducing toxicity and unwanted side effects—a major challenge in conventional anticancer therapy.

Bar chart showing CI values at different concentration ratios of APE (1, 1/2, and 1/4 × IC50) combined with CRE (1, 1/2, and 1/4 × IC50). CI < 1 indicates synergism, CI = 1 indicates an additive effect, and CI > 1 indicates antagonism. Synergistic effects are observed particularly at lower concentration ratios (APE 1/4 + CRE 1/2, CI = 0.6; APE 1/4 + CRE 1/4, CI = 0.4).

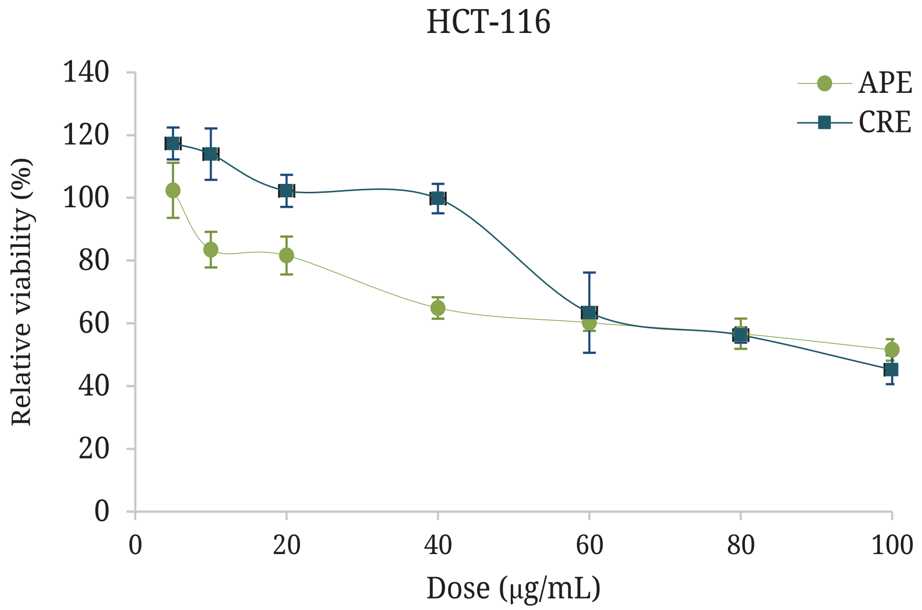

FIGURE 1. Results of MTT assay to compare cell viability following treatment with APE and CRE for 24 h in HCT116 cell line.

TABLE 1. The CI value description

Value range	Description	Graded symbols
< 0.1	Very strong synergism	+++++
0.1-0.3	Strong synergism	++++
0.3-0.7	Synergism	+++
0.7-0.85	Moderate synergism	++
0.85-0.9	Slight synergism	+
0.9-1.1	Nearly additive	±
1.2-1.45	Slight antagonism	-
1.45-3.3	Antagonism	--
3.3-10	Strong antagonism	---
>10	Very strong antagonism	----

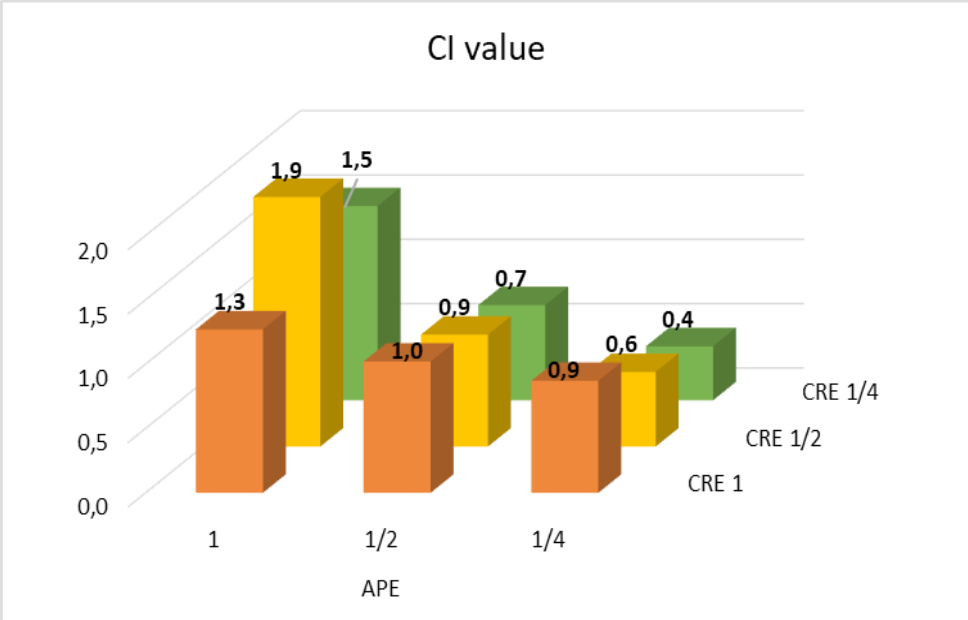

FIGURE 2. Combination Index (CI) values of *Andrographis paniculata* extract (APE) and *Catharanthus roseus* extract (CRE) against HCT-116 cells.

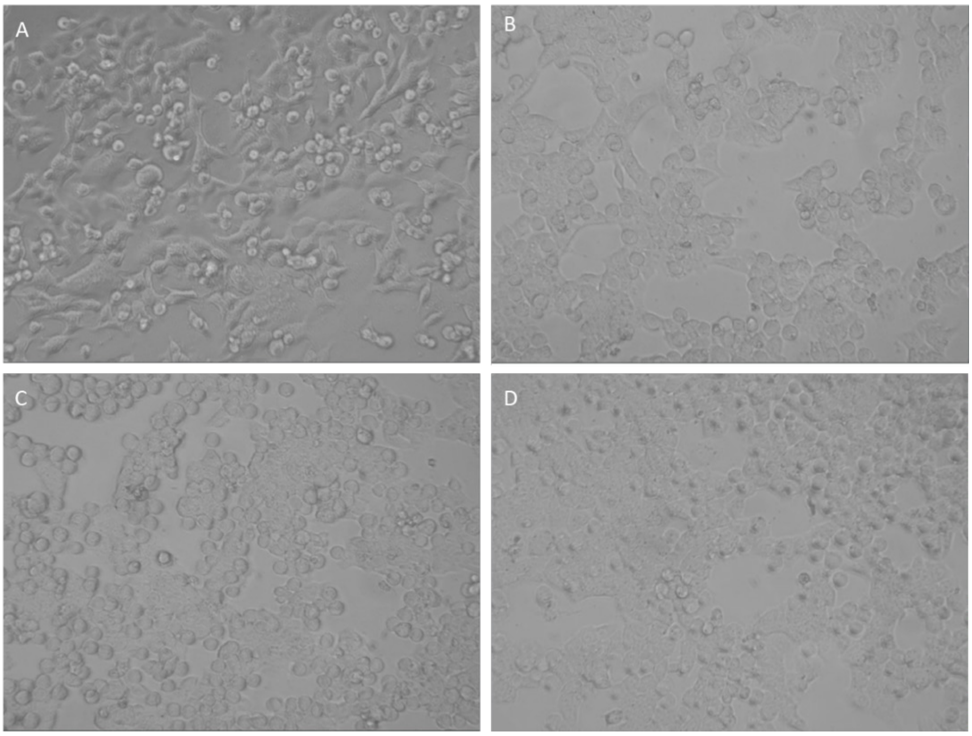

FIGURE 3. Morphological changes of HCT-116 cells following treatment with *Andrographis paniculata* extract (APE) and *Catharanthus roseus* extract (CRE). Mag 200x

Representative phase-contrast microscopy images show untreated control cells (upper left) and cells treated with different concentration combinations of APE and CRE: (A) untreated control, (B) APE 90.1 μg/mL + CRE 89.6 μg/mL, (C) APE 22.5 μg/mL + CRE 44.8 μg/mL, and (D) APE 22.5 μg/mL + CRE 22.4 μg/mL. Images were captured at magnification 200×.

DISCUSSION

This study demonstrated that both APE and CRE possess significant cytotoxic effects against HCT-116 cells, with comparable IC_{50} values of approximately 90 μg/mL. More importantly, the combination of APE and CRE resulted in a synergistic effect at several concentration ratios, particularly at one-quarter of the IC_{50} of both extracts, where the CI:0.42. These findings suggest that phytotherapeutic combinations can enhance anticancer activity while reducing the required dosage, which may minimize toxicity and improve therapeutic outcomes.

The cytotoxic activity of APE is consistent with previous reports describing andrographolide, its major diterpenoid lactone, as a potent anticancer agent. Andrographolide has been shown to inhibit proliferation, migration, and invasion of colorectal cancer cells by suppressing PI3K/Akt and NF-κB signaling, inducing apoptosis, and promoting ferroptosis.[18,19] In line with this, previous study reported that andrographolide effectively suppressed tumor growth and metastasis in CRC models by downregulating Wnt/β-catenin signaling.[19] Similarly, CRE contains bioactive vinca alkaloids such as vincristine and vinblastine, which disrupt microtubule polymerization, leading to mitotic arrest and apoptosis.[20] These mechanisms support our observation that CRE alone demonstrated strong cytotoxic activity against HCT-116 cells. Interestingly, when combined, APE and CRE exhibited synergistic interactions, particularly at sub-toxic doses. The strongest synergy (CI = 0.58) observed when both extracts were administered at ¼ IC_{50} concentrations. This is consistent with the principle that phytochemical combinations can target multiple cancer hallmarks simultaneously, leading to enhanced efficacy.[21] For example, andrographolide modulates intracellular signaling and redox homeostasis, while vinca alkaloids directly disrupt cell division.[22,23] Their complementary actions likely underlie the observed synergism. Comparable findings were reported by Ibrahim *et al.*,[13] who showed that combined plant extracts enhanced apoptosis induction in colorectal cancer cells through cooperative modulation of intrinsic and extrinsic apoptotic pathways.

The capacity of APE and CRE to exhibit synergistic cytotoxicity at lower dosages is extremely significant from a translational standpoint.[24] Conventional chemotherapy for CRC is often limited by systemic toxicity, chemoresistance, and patient intolerance.[25] By lowering the required effective concentrations, combination phytotherapy may provide an opportunity to reduce adverse effects while maintaining or even enhancing anticancer efficacy.[26] Such an approach aligns with growing evidence that integrating natural compounds into standard treatment regimens can sensitize tumor cells to chemotherapy and overcome resistance.[27,28] Nevertheless, several limitations should be noted. First, this study was restricted to in vitro assays on a single colorectal cancer cell line, which may not fully represent tumor heterogeneity or in vivo complexity.[29] The tumor microenvironment, pharmacokinetics, and potential systemic interactions could significantly influence therapeutic outcomes.[30] Second, the crude extracts contain multiple bioactive constituents beyond andrographolide and vinca alkaloids, and their individual or interactive contributions remain unclear.[31] Advanced phytochemical characterization and mechanistic studies are needed to clarify the precise molecular targets of this combination.[32] Finally, the lack of normal colon epithelial cell assays limits our ability to assess selectivity and safety.[33]

Future research should focus on in vivo validation using colorectal cancer animal models to evaluate efficacy, toxicity, and pharmacodynamics. Investigating nanoparticle-based delivery systems may further enhance the bioavailability of andrographolide and vinca alkaloids.[34] Moreover, exploring combinations with conventional chemotherapeutics such as 5-fluorouracil could provide clinically translatable insights into the role of APE and CRE as adjuvants in CRC management.[35]

CONCLUSION

This study provides preliminary evidence that *Andrographis paniculata* and *Catharanthus roseus* extracts exert synergistic cytotoxic effects against colorectal cancer HCT-116 cells. Their combination allows dose reduction while maintaining strong anticancer activity, highlighting their potential as a complementary phytotherapeutic strategy for CRC. Further mechanistic and in vivo studies are warranted to validate these promising findings.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

AUTHOR CONTRIBUTION

FNPR: Methodology, Investigation, Data Analysis, Writing – Original Draft. **NH:** Conceptualization, Project Administration, Supervision, Writing – Review & Editing. **AP:** Laboratory Work, Data Analysis, Visualization. **LNP:** Formal Analysis, Literature Review, Writing – Review & Editing.

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