

Study on Antioxidant and Cytotoxicity Activities of Fractions from *Coptosapelta Flavescens* Korth. Rubiaceae in Human Breast Cancer MDA-MB-231 Cell Line

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Abstract

Coptosapelta flavescens has been reported to possess various biological activities, particularly antioxidant and cytotoxicity effects. This study evaluated the antioxidant and cytotoxic properties of fractionated extracts against the MDA-MB-231 breast cancer cell line. The fractions were obtained by solvent partitioning with increasing polarity, namely n-hexane, chloroform, dichloromethane, ethyl acetate, and methanol. Total polyphenol content was determined using the Folin–Ciocalteu method, antioxidant activity by the 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH), and cytotoxicity by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT). Polyphenols were primarily distributed in the ethylacetate, chloroform, and dichloromethane fractions, with their concentrations of (186.65, 211.95, and 219.66) $\mu\text{g GAE/mg}$, respectively. These fractions also exhibited antioxidant capacity with IC_{50} values of (15.24, 12.27, and 11.59) $\mu\text{g/mL}$, respectively. Furthermore, they demonstrated cytotoxic activities against MDA-MB-231 cells, reducing cell viability by 50% after 72 h at concentrations of (88.12, 106.67, and 104.72) $\mu\text{g/mL}$, respectively. These findings indicate that these fractions are promising sources of bioactive compounds with antioxidant and cytotoxic potential.

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1 Introduction

Cancer is currently recognized as one of the most pressing global health concerns worldwide. According to the International Agency for Research on Cancer (IARC), an estimated 19.2 million new cancer cases were reported worldwide in 2020 [1]. Among cancer types, breast cancer is the second most prevalent after lung cancer and remains one of the top five causes of cancer-related mortality [2]. In Viet Nam, the incidence of breast cancer

has been steadily rising, with an increasing trend toward younger age at onset. The disease occurs across nearly all post-pubertal age groups, social strata, and occupational backgrounds, underscoring its broad public health impact. Breast cancer is conventionally treated with surgery, radiotherapy, chemotherapy, and hormone therapy. While these modalities improve survival, they are limited by high treatment costs – especially in Viet Nam, where most drugs are imported – and by adverse effects such as



hematological toxicity and alopecia, which impair quality of life [3]. These issues underscore the need for affordable, safer, and more effective alternatives, including plant-derived agents with anticancer potential. Polyphenols, widely present in herbal sources, act as natural antioxidants that scavenge free radicals, modulate nitric oxide activity, and exert antiproliferative and phytoestrogenic effects, offering considerable potential for cancer prevention and therapy [4-6].

Coptosapelta flavescens Korth., Rubiaceae (CF) is a promising medicinal plant in Viet Nam, particularly abundant in the south, with notable pharmacological potential. For a long time, ethnic minorities in Ninh Thuan province (now part of Khanh Hoa province) have used this herb to create a traditional remedy called "Cao Khai" – an aqueous extract, which was utilised for wound cleansing, antimicrobial, and anti-inflammatory purposes. Recent studies have identified key constituents from the roots of *C. flavescens*, including 3 β -O- β -D-quinovopyranosid pyrocincholic acid, acid pyroquinoic

[7] and several anthraquinones [9,67]1-hydroxyl-2-ethoxycarbonyl anthraquinon, 1-hydroxyl-2- methyl -4methoxy-anthraquinon, 1-hydroxyl-2-hydroxymethyl-anthraquinon [8]. In 2015, the cytotoxicity of anthraquinones and root extracts against the RD-A cell line was documented, and 1-hydroxyl-2-ethoxycarbonyl anthraquinone showed an IC₅₀ of 9.37 μ M. [8]. These findings highlight *C. flavescens* as a promising source of bioactive compounds with potential anticancer activity, including applications in breast cancer. Therefore, this research was conducted to screen the antioxidant and cytotoxic activities of the CF's fractions with three specific objectives: (1) to determine the total polyphenol content of CF's fractions using the Folin-Ciocalteu method, (2) to evaluate CF's fractions antioxidant activity by the DPPH assay, (3) to assess CF's fractions cytotoxic effects on the MDA-MB-231 breast cancer cell line.

2 Materials and Methods

2.1 Materials

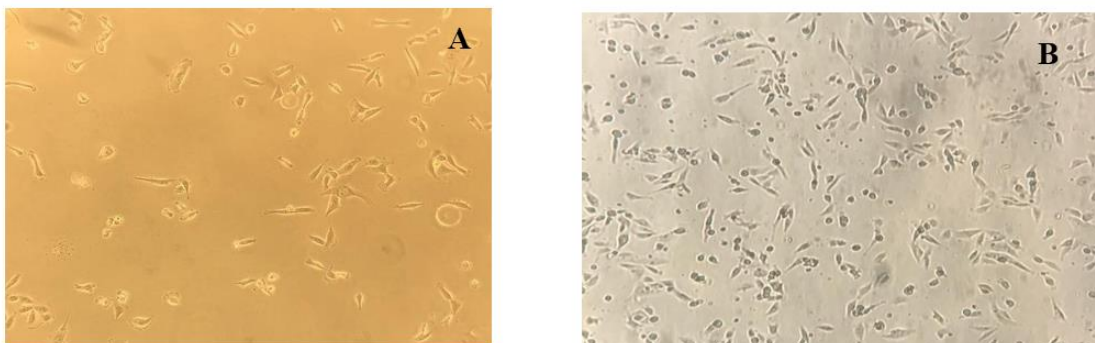


Figure 1 Morphology of MDA-MB-231 cells under inverted microscopy (20X)

A: 2 days after subculture in a 75 cm² flask; B: 1 day after seeding in a 96-well plate at (1.5×10^4) cells/cm²

Samples: six fraction extracts of *C. flavescens* – total (CK), n-hexane (n-hex), chloroform (CHCl₃), dichloromethane (CH₂Cl₂), ethyl acetate (EA), and methanol (MeOH) – were obtained through solvent partitioning with increasing polarity as previously reported [9].

Cell lines: the human breast cancer cell line MDA-MB-231 was sourced from the American Type Culture Collection (ATCC, USA). Cell preservation and culture procedures were performed at the Faculty of Pharmacy, University of Medicine and Pharmacy, Ho Chi Minh City. MDA-MB-231 is a human breast

adenocarcinoma cell line derived from a 51-year-old Caucasian female. With a 38-hour doubling time, it expresses EGF and TGF- α receptors, carries the WNT7B oncogene, and is widely used in *in vitro* models to predict *in vivo* toxicity.

Chemicals and reagents: Dulbecco's Modified Eagle Medium (DMEM), fetal calf serum (FCS), L-glutamine, penicillin-streptomycin, trypsin-EDTA, MTT, dimethyl sulfoxide (DMSO), doxorubicin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, gallic acid monohydrate, quercetin, methanol,

isopropanol, hydrochloric acid (HCl), phosphate-buffered saline (PBS).

Equipment: Analytical Balance (Mettler Toledo), 96-Well Culture Plates, Inverted microscope, UV-Vis Spectrophotometer, Elisa Microplate Reader, Vortex Mixer (VX-200), micropipettes (1 μ L to 10 μ L and 100 μ L to 1000 μ L), Autoclave, CO₂ Incubator, Biosafety Cabinet (AC2-4E1).

2.2 Methods

2.2.1 Determination of total phenol content of CF's fraction extracts

The total phenol content (TPC) was determined using the Folin-Ciocalteu reagent assay [10]. A volume of 50 μ L of each fraction was mixed with 250 μ L of 10% Folin-Ciocalteu reagent in 1.5 mL tubes and reacted for 5 min at room temperature. Subsequently, a volume of 200 μ L of 7.5% Na₂CO₃ was added, and the mixture was incubated in the dark for 1 h. After incubation, the reaction mixture was transferred to a 96-well plate, and absorbance was measured at 765 nm. Each experiment was performed in triplicate, and the mean values were calculated.

Gallic acid served as the standard, and the total polyphenol content was expressed as μ g gallic acid equivalents per mg extract (μ g GAE/mg) according to the following equation:

$$\text{TPC} = (C \times V \times K \times \text{purity})/m \quad (1)$$

Where:

TPC: total polyphenol content (μ g GAE/mg extract)

C: x value obtained from the gallic acid calibration curve (μ g/mL)

V: volume of the sample extract (mL)

K: dilution factor

m: mass of the extract present in the tested volume (mg)

The purity of gallic acid used in the experiment was 0.98.

2.2.2 Determination of total antioxidant capacity (TAC)

Antioxidant activity was initially screened by thin-layer chromatography (TLC) on silica gel 60 F₂₅₄ plates. Six CF fractions and quercetin (1,000 μ g/mL) were spotted (10 μ L), allowed to dry, and briefly immersed in 1 mM DPPH solution for 10 s. Fractions exhibiting yellow spots on a purple background were considered active and selected for subsequent IC₅₀ determination.

Determination of IC₅₀ value of potential fractions using the DPPH radical scavenging assay [11]: Potential fractions were dissolved in appropriate solvents at 1000 μ g/mL and further diluted to concentrations ranging from (0-20) μ g/mL. A volume of 40 μ L of each potential fraction was mixed with 160 μ L of 0.1 mM DPPH in a 96-well plate and incubated in the dark for 30 min. Absorbance was measured at 517 nm. Experiments were performed in triplicate, and mean values were calculated. Quercetin in methanol was used as a positive control. The percentage of DPPH radical scavenging activity was calculated using the following formula (2). Based on the results, a dose-response curve was constructed, from which the IC₅₀ values were determined.

$$\% \text{HTCO} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \quad (2)$$

Where

A sample: absorbance of the CF's fractions or the quercetin mixed with the DPPH solution

A control: absorbance of the DPPH solution

2.2.3 *In vitro* Evaluation of cytotoxic activity of CF's fractions on MDA-MB-231 Breast Cancer Cells

Cell viability of MDA-MB-231 cells was evaluated using the MTT assay [12], which relies on mitochondrial succinate dehydrogenase activity. CF's fractions were prepared in 1% DMSO and diluted to (25-200) μ g/mL in culture medium. After 72 h treatment, cells were incubated with 0.5 mg/mL MTT in serum-free medium at 37 °C, 5% CO₂ for (3-4) h. Formazan crystals were dissolved in acidified isopropanol, and absorbance at 570 nm was measured. Cell viability inhibition was calculated as described in formula (3). Based on the results, IC₅₀ values were determined from dose-response curves of percentage inhibition versus extract concentration. Each experiment was performed in triplicate, and the mean values were calculated.

$$\% \text{inhibition} = \frac{A_{\text{negative control}} - A_{\text{sample}}}{A_{\text{negative control}}} \times 100 \quad (3)$$

Where

A_{sample}: absorbance of the CF's fractions or the doxorubicin-treated group



$A_{\text{negative control}}$: absorbance of the negative control group treated with the sample solvent diluted in culture medium

2.2.4 Statistical analysis

The results are shown as mean SD (standard deviation of mean). Pearson correlation analysis was performed using SPSS 22.0 to evaluate the relationship between antioxidant activity and total polyphenol content in these samples.

3 Results and Discussion

3.1 Contents of total polyphenol compounds

Absorbance values at 765 nm of gallic acid standards at concentrations ranging from (1 to 10) $\mu\text{g/mL}$ are presented in Table 1.

Based on these data, a correlation between absorbance and gallic acid concentration was constructed, with the

regression equation $y = 0.0381x + 0.0088$ and a correlation coefficient of $R^2 = 0.9994$, where y represents absorbance and x represents gallic acid concentration ($\mu\text{g/mL}$).

Table 1 Concentration and absorbance of gallic acid standards (Mean \pm SD)

Concentration of GAE ($\mu\text{g/mL}$)	Absorbance (MEAN \pm SD)	Concentration of GAE ($\mu\text{g/mL}$)	Absorbance (MEAN \pm SD)
1	0.044 \pm 0.002	6	0.241 \pm 0.004
2	0.083 \pm 0.002	7	0.273 \pm 0.003
3	0.126 \pm 0.003	8	0.310 \pm 0.002
4	0.164 \pm 0.002	9	0.351 \pm 0.002
5	0.201 \pm 0.004	10	0.390 \pm 0.006

Absorbance values at 765 nm of six fractions are presented in Table 2.

Table 2 Concentration and absorbance of six fractions (Mean \pm SD)

Samples	Concentration ($\mu\text{g/mL}$)	Absorbance (MEAN \pm SD)	Samples	Concentration ($\mu\text{g/mL}$)	Absorbance (MEAN \pm SD)
Total (CK)	100	0.177 \pm 0.003	CH_2Cl_2	30	0.265 \pm 0.003
n-hex		0.328 \pm 0.009	CHCl_3		0.256 \pm 0.004
MeOH		0.213 \pm 0.008	EA		0.2265 \pm 0.0005

Using this regression equation, the x values were determined and then substituted into the formula described in section 2.2.1; TPC of each fraction ($\mu\text{g GAE/mg}$) was calculated, as presented in Table 3.

Table 3 TPC of the total extract and all six fractions

Samples	TPC ($\mu\text{g GAE/mg}$)	Samples	TPC ($\mu\text{g GAE/mg}$)
Total (CK)	43.26	CH_2Cl_2	219.66
n-hex	82.10	CHCl_3	211.95
MeOH	52.52	EA	186.65

TPC of the extracts ranged from (43.26 to 219.66) $\mu\text{g GAE/mg}$, with all fractionated extracts higher than the total extract. The order of TPC from lowest to highest was $\text{CK} < \text{MeOH} < \text{n-hex} < \text{EA} < \text{CHCl}_3 < \text{CH}_2\text{Cl}_2$. Notably, three fractions - EA, CHCl_3 , and CH_2Cl_2 - showed comparable high polyphenol content (186.65, 211.95 and 219.66) $\mu\text{g GAE/mg}$, respectively). Polyphenols are plant compounds with hydroxyl groups on benzene rings, which confer antioxidant properties. Their content is positively correlated with radical-scavenging activity, helping protect the body from oxidative stress [10]. These findings indicated that the CHCl_3 , CH_2Cl_2 and EA fractions had high antioxidant potential and were therefore selected for further evaluation using *in vitro* DPPH radical scavenging assay.

3.2 Antioxidant potential

The preliminary antioxidant screening of the fractions on silica gel 60 F_{254} TLC plates is shown in Figure 2. Four yellow spots on a purple background (spots 1, 4, 5, and 6) after dipping the silica gel 60 F_{254} TLC plate in 1 mM DPPH, corresponding to quercetin, CH_2Cl_2 , CHCl_3 , and EA fractions, indicating their stronger *in vitro* antioxidant activity compared to the other fractions. In contrast, n-hex, MeOH, CK, and the MeOH solvent control showed no visible activity at 1,000 $\mu\text{g/mL}$. Based on these findings, the CHCl_3 , CH_2Cl_2 , and EA fractions were selected for IC_{50} determination using the DPPH radical scavenging assay.

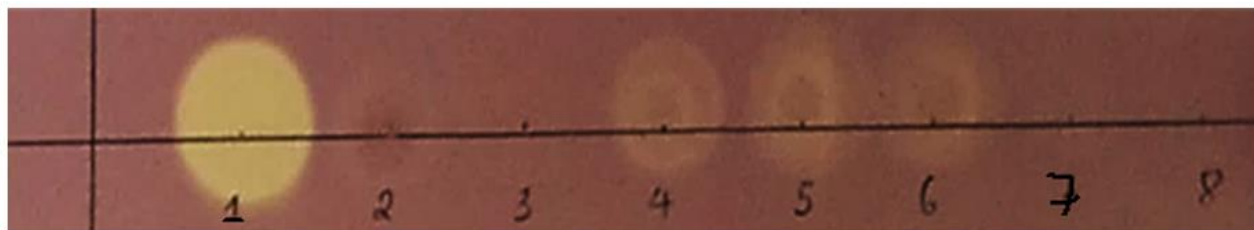


Figure 2 The preliminary antioxidant screening of the fractions

Note: (1) Quercetin; (2) CK; (3) n-hex; (4) CHCl_3 ; (5) CH_2Cl_2 ; (6) EA; (7) MeOH; (8) MeOH solvent

The *in vitro* antioxidant activity of the three potential fractions at concentrations of (2-20) $\mu\text{g/mL}$ and the reference compound quercetin at (0.8-4) $\mu\text{g/mL}$, assessed using the DPPH assay, is presented in Table 4.

Table 4 *In vitro* antioxidant activity of quercetin and CF's potential fractions (Mean \pm SD)

Samples	Concentration $\mu\text{g/mL}$	HTCO (%)	Samples	Concentration $\mu\text{g/mL}$	HTCO (%)
CH_2Cl_2	2	28.75 \pm 1.75	EA	2	29.30 \pm 0.09
	4	31.41 \pm 0.96		4	31.17 \pm 1.09
	8	40.94 \pm 1.95		8	37.28 \pm 0.79
	12	49.83 \pm 3.01		12	44.46 \pm 0.85
	16	60.39 \pm 1.73		16	51.85 \pm 1.21
	20	70.79 \pm 4.65		20	57.79 \pm 0.96
CHCl_3	2	30.14 \pm 0.69	Quercetin	0.8	34.80 \pm 0.89
	4	31.06 \pm 1.87		1.6	42.85 \pm 1.75
	8	43.82 \pm 3.21		2.4	50.14 \pm 3.04
	12	51.85 \pm 0.17		3.2	60.10 \pm 3.40
	16	55.14 \pm 2.08		4	66.65 \pm 3.13
	20	65.13 \pm 0.17			

The antioxidant activity of all fractions increased proportionally with extract concentration. Specifically, quercetin showed DPPH radical scavenging activity rising from 34.80% at 0.8 $\mu\text{g/mL}$ to 66.65% at 4 $\mu\text{g/mL}$. The CHCl_3 fraction exhibited an increase from 30.14% at 2 $\mu\text{g/mL}$ to 65.13% at 20 $\mu\text{g/mL}$, CH_2Cl_2

from 28.75% to 70.79%, and EA from 29.30% to 57.79% over the same concentration range.

Based on the obtained results in Table 4, dose-response curves were plotted in Microsoft Excel to establish the linear regression equations ($y = ax + b$), from which IC_{50} values were calculated by substituting $y = 50$, as presented in Table 5.

Table 5 IC_{50} values of quercetin and CF's potential fractions

Sample	Linear Equation	R^2	IC_{50} ($\mu\text{g/mL}$)
Quercetin	$y = 10.119x + 26.624$	$R^2 = 0.9969$	2.31
CH_2Cl_2 fraction	$y = 2.365x + 22.579$	$R^2 = 0.9964$	11.59
CHCl_3 fraction	$y = 1.9656x + 25.878$	$R^2 = 0.9764$	12.27
EA fraction	$y = 1.6356x + 25.073$	$R^2 = 0.9965$	15.24

Compared to quercetin, the CHCl_3 and CH_2Cl_2 fractions of CF showed notable antioxidant activity ($\text{IC}_{50} = 12.27$ $\mu\text{g/mL}$ and 11.59 $\mu\text{g/mL}$), about six times higher than

quercetin ($\text{IC}_{50} = 2.31$ $\mu\text{g/mL}$), while the EA fraction was weaker ($\text{IC}_{50} = 15.24$ $\mu\text{g/mL}$), with potency ranking $\text{EA} < \text{CHCl}_3 < \text{CH}_2\text{Cl}_2$. Although less potent than quercetin,

these fractions still demonstrate significant potential as natural antioxidants, which may help mitigate oxidative stress – a key factor in DNA damage, chronic inflammation, and cancer development.

Previous studies reported a positive correlation between antioxidant activity and anticancer effects [13]. Chronic oxidative stress damages biomolecules, lipid peroxides, proteins, enzymes, and DNA, disrupting redox homeostasis and contributing to cancer development. Preventive strategies include minimizing free radical generation, enhancing antioxidant intake, maintaining a healthy lifestyle (avoiding smoking and alcohol, reducing chemical exposure, exercising regularly), and proper nutrition [13]. Some environmental carcinogens can directly generate free radicals and activate inflammatory cells to produce ROS and RNS *in vivo* [13]. Thus, the potent antioxidant activity of *C. flavescens* fractions suggests potential for preventing oxidative stress-related diseases and supporting cancer therapy. Beyond scavenging ROS and protecting DNA, promoting apoptosis in cancer cells is a current therapeutic strategy. Therefore, this study further evaluated the cytotoxic effects of the fractions *in vitro* using the MTT assay on MDA-MB-231 breast cancer cells.

3.3 Correlation between polyphenol content and antioxidant activity

Pearson’s correlation coefficient (*r*) was used to evaluate the relationship between two quantitative variables. Values of *r* approaching 1 or -1 indicate a strong linear correlation: positive when close to 1,

Table 7 Cytotoxic activity of these fractions against human breast cancer MDA-MB-231 cells at a concentration of 200 µg/mL after 72 h of treatment.

Fractions	CK	MeOH	CHCl ₃	CH ₂ Cl ₂	EA	n-hex
% inhibition	24.30 ± 0.66	19.87 ± 0.87	82.34 ± 0.92	87.48 ± 0.48	85.27 ± 0.61	75.65 ± 0.69

As shown in Table 7, at the concentration of 200 µg/mL, the dichloromethane (CH₂Cl₂), ethyl acetate (EA), chloroform (CHCl₃), and n-hexane (n-hex) fractions exhibited strong cytotoxic activity against MDA-MB-231 cells, with inhibition rates of 87.48%, 85.27%, 82.34%, and 75.65%, respectively. In contrast, the crude extract (CK) and methanol (MeOH) fractions showed relatively weak inhibitory effects, with inhibition rates of 24.30% and 19.80%, respectively, compared with the negative control (DMSO). Accordingly, the cytotoxicity assay was performed

negative when close to -1. Statistical significance was assessed, with *p* < 0.05 indicating a significant linear correlation.

The correlation between polyphenol content and IC₅₀ values of the CHCl₃, CH₂Cl₂, and EA fractions was determined using SPSS and is shown in Table 6.

Table 6 Pearson correlation between polyphenol content and IC₅₀ of CF’s potential fractions

Pearson correlation (<i>r</i>)	Polyphenol	IC ₅₀
Polyphenol	1	-0.999**
IC ₅₀	-0.999**	1

***p* < 0.01, statistically significant

As shown in Table 6, *r* = -0.999 with *p* < 0.01 indicates a strong, statistically significant negative correlation. This means that higher TPC corresponds to lower IC₅₀ values and stronger DPPH radical scavenging activity, suggesting that polyphenol content largely determines the antioxidant potential of the tested CF fractions.

3.4 Assessment of the cytotoxic activity of CF’s fractions on the MDA-MB-231 human breast cancer cell line

Evaluation of the growth-inhibitory effect of CF’s fractions on MDA-MB-231 cells at 200 µg/mL.

The results of the MTT assay evaluating cell viability after 72 h of treatment with these fractions are presented in Table 7.

further on the four most promising fractions (CHCl₃, CH₂Cl₂, EA, n-hex fractions) to determine their IC₅₀ values, defined as the concentration at which 50% cell growth is inhibited.

3.4.1 Determination of the IC₅₀ values of CF’s potential fractions against human breast cancer MDA-MB-231 cells

The growth-inhibitory effects of the four most promising fractions and reference of doxorubicin relative to the negative control are summarized in Table 8 and Table 9.



Table 8 Cytotoxic activity and IC₅₀ values of the four most promising fractions against MDA-MB-231 breast cancer cells after 72 h of treatment

Fractions	Inhibition rate compared with negative control (%)				Regression equation	R ²	IC ₅₀ (µg/mL)
	200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL			
CHCl ₃	82.34 ± 0.92	46.20 ± 0.94	21.24 ± 0.71	4.65 ± 0.57	$y = -0.0011x^2 + 0.6938x - 11.5$	0.9997	106.67
CH ₂ Cl ₂	87.48 ± 0.48	48.09 ± 0.71	26.00 ± 1.18	12.71 ± 1.26	$y = -0.0005x^2 + 0.5287x + 0.1176$	0.9998	104.72
EA	85.27 ± 0.61	52.84 ± 0.72	39.83 ± 0.59	26.11 ± 0.61	$y = -0.0003x^2 + 0.3866x + 18.261$	0.9931	88.12
n-hex	75.65 ± 0.69	31.99 ± 0.45	14.07 ± 1.15	9.91 ± 0.19	$y = 0.0008x^2 + 0.1895x + 3.849$	0.9989	149.36

Based on the dose-response data, the IC₅₀ values of the four fractions and doxorubicin were calculated (Table 8 and Table 9). Among them, the EA fraction exhibited the strongest cytotoxic effect with an IC₅₀ of 88.12 µg/mL, followed by CH₂Cl₂ (104.72 µg/mL) and

CHCl₃ (106.67 µg/mL). In contrast, the n-hexane fraction showed weaker activity with an IC₅₀ of 149.36 µg/mL. In comparison, the standard chemotherapeutic agent doxorubicin showed markedly stronger activity, with an IC₅₀ value of 1.2 µM.

Table 9 Cytotoxic activity and IC₅₀ values of the reference doxorubicin against MDA-MB-231 breast cancer cells after 72 hours of treatment

Fractions	Inhibition rate compared with negative control (%)				Regression equation	R ²	IC ₅₀ µM
	5 µM	2.5 µM	1 µM	0.5 µM			
Doxorubicin	75.49 ± 0.32	71.32 ± 0.46	46.72 ± 1.04	31.91 ± 0.49	$y = -4.0224x^2 + 31.611x + 17.917$	0.9981	1.2

Overall, the CH₂Cl₂, CHCl₃, and EA fractions consistently demonstrated high polyphenol contents, strong antioxidant activity, and moderate cytotoxicity against MDA-MB-231 cells. However, these findings should be considered preliminary because the study was limited to a single cancer cell line and did not include a non-cancerous control cell line. When compared with previous studies, these IC₅₀ values indicate a moderate cytotoxic effect. For example, in 2014, isolated anthraquinone derivatives from *C. flavescens* were reported to exhibit lower IC₅₀ values against Vero cells, ranging from (7.1 to 48.9) µg/mL [14]. Similarly, 1-hydroxyethoxycarbonyl-anthraquinone and 1-hydroxy-2-hydroxymethyl-anthraquinone exhibited cytotoxic effects against RD-A cells with IC₅₀ values of 9.371 µM and 92.66 µM, respectively.

The observed cytotoxicity may be associated with the presence of polyphenols, triterpenoids, anthraquinones,

and saponins previously reported in *C. flavescens* [7,15]. In particular, the CH₂Cl₂, CHCl₃, and EA fractions contained high levels of polyphenols and showed strong antioxidant activity, suggesting that these fractions may contribute to cancer cell inhibition partly through the reduction of oxidative stress. Excessive production of reactive oxygen species (ROS) can disrupt the structure of proteins, lipids, lipoproteins, and DNA, leading to a cascade of pre-cancerous events such as cell cycle promotion, gene mutation, abnormal gene expression, cellular senescence, and necrosis [4]. ROS may directly damage DNA or activate aberrant signaling pathways that enhance cancer cell survival [5-6]. Therefore, inhibiting or scavenging ROS is considered an important therapeutic strategy for cancer. These findings suggest that the CH₂Cl₂, CHCl₃, and EA fractions are promising sources of polyphenol-rich compounds with strong antioxidant activity and

potential anticancer effects, providing an initial basis for further screening and mechanistic studies.

4 Conclusions

This research initially screened that polyphenols were enriched in the ethylacetate, chloroform, and dichloromethane fractions of *C. flavescens* Korth (ranging from (186.65 to 219.66) $\mu\text{g GAE/mg}$), which showed strong antioxidant activity ($\text{IC}_{50} = (11.59-15.24) \mu\text{g/mL}$) and notable cytotoxicity against MDA-MB-

231 cells ($\text{IC}_{50} = (88.12-106.67) \mu\text{g/mL}$). These results suggest that these fractions are promising sources of bioactive compounds with dual antioxidant and anticancer potential, warranting further phytochemical and mechanistic studies.

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Đánh giá hoạt tính kháng oxy hóa và độc tế bào trên dòng tế bào ung thư vú MDA-MB-231 của các phân đoạn Dây khai

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Tóm tắt Dây khai (*Coptosapelta flavescens*) được báo cáo có nhiều hoạt tính sinh học, đặc biệt là khả năng chống oxy hóa và độc tế bào. Nghiên cứu này nhằm đánh giá hoạt tính chống oxy hóa và độc tế bào của các cao phân đoạn từ dây khai trên dòng tế bào ung thư vú MDA-MB-231. Các phân đoạn được thu bằng phương pháp lactic phân bố với các dung môi có độ phân cực tăng dần, bao gồm n-hexan, chloroform, dichloromethan, ethylacetat và methanol. Hàm lượng polyphenol được xác định theo phương pháp Folin-Ciocalteu, khả năng chống oxy hóa bằng phương pháp DPPH, và hoạt tính độc tế bào bằng phương pháp MTT. Polyphenol tập trung chủ yếu ở các phân đoạn ethylacetat, chloroform và dichloromethan với hàm lượng tương ứng (186,65; 211,95 và 219,66) $\mu\text{g GAE/mg}$. Các phân đoạn này thể hiện khả năng chống oxy hóa với giá trị IC_{50} lần lượt là (15,24; 12,27 và 11,59) $\mu\text{g/mL}$ và thể hiện hoạt tính độc tế bào trên dòng MDA-MB-231, làm giảm 50 % tỷ lệ sống của tế bào sau 72 giờ ở các nồng độ tương ứng là (88,12; 106,67 và 104,72) $\mu\text{g/mL}$. Kết quả cho thấy các phân đoạn ethylacetat, chloroform và dichloromethan là nguồn hợp chất sinh học tiềm năng với hoạt tính chống oxy hóa và độc tế bào, làm cơ sở cho các nghiên cứu phân lập và xác định cấu trúc hợp chất trong tương lai.

Từ khóa Chống oxy hóa; *Coptosapelta flavescens*; độc tế bào; MDA-MB-231; polyphenol.