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Cytotoxic Interaction Between Cisplatin and *Eclipta alba* Extract in HeLa Cervical Cancer Cells

Interaksi Sitotoksik antara Cisplatin dan Ekstrak *Eclipta alba* pada Sel Kanker Serviks HeLa

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ABSTRACT

Cervical cancer remains a major cause of cancer-related mortality among women worldwide. Cisplatin is a first-line chemotherapeutic agent, but its use is limited by resistance and dose-dependent toxicity. This study evaluated the cytotoxic interaction between cisplatin and *Eclipta alba* extract in HeLa cervical cancer cells. Ethanolic maceration yielded 17.38 g of extract, corresponding to 10.22%. Thin-layer chromatographic screening indicated flavonoids, coumarins, and triterpenoid saponins, while LC–MS/MS tentatively identified eclalbasaponin I, wedelolactone, pinoquercetin, luteolin, and quercitrin. Cytotoxicity was assessed using the MTT assay. The IC_{50} values were 1.15 ± 0.16 µg/mL for cisplatin and 598.29 ± 42.89 µg/mL for the extract. Under fixed sub-IC_{50} cisplatin conditions, 0.42 µg/mL, the extract IC_{50} decreased to 64.97 ± 5.41 µg/mL. Combination index values below 1 suggested synergistic interaction under fixed-dose conditions, indicating a potential dose-sparing strategy requiring further validation.

Kata kunci:
Cisplatin
Efek sinergis
Eclipta alba
Kanker serviks
Sel HeLa

ABSTRAK

Kanker serviks masih menjadi salah satu penyebab utama kematian akibat kanker pada perempuan di seluruh dunia. Cisplatin merupakan agen kemoterapi lini pertama, tetapi penggunaannya dibatasi oleh resistensi dan toksisitas yang bergantung pada dosis. Penelitian ini mengevaluasi interaksi sitotoksik antara cisplatin dan ekstrak *Eclipta alba* pada sel kanker serviks HeLa. Maserasi etanol menghasilkan 17,38 g ekstrak dengan rendemen 10,22%. Skrining kromatografi lapis tipis menunjukkan adanya flavonoid, kumarin, dan saponin triterpenoid, sedangkan LC–MS/MS secara tentatif mengidentifikasi eclalbasaponin I, wedelolakton, pinoquercetin, luteolin, dan quercitrin. Sitotoksisitas dievaluasi menggunakan uji MTT. Nilai IC_{50} cisplatin dan ekstrak masing-masing sebesar 1,15 ± 0,16 µg/mL dan 598,29 ± 42,89 µg/mL. Pada kondisi cisplatin sub-IC_{50} tetap, 0,42 µg/mL, IC_{50} ekstrak menurun menjadi 64,97 ± 5,41 µg/mL. Nilai indeks kombinasi <1 menunjukkan interaksi sinergis pada kondisi dosis tetap, sehingga mengindikasikan potensi strategi penghematan dosis yang masih memerlukan validasi lebih lanjut.

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1. INTRODUCTION

Cervical cancer remains a major global health burden and continues to contribute substantially to cancer-related mortality among women. Persistent infection with high-risk human papillomavirus (HPV), particularly types 16 and 18, is recognized as the principal etiological factor in cervical carcinogenesis because it promotes cellular transformation in the cervical squamous epithelium (Nabi et al., 2025). Globally, cervical cancer is the fourth most common cancer among women, with approximately 660,000 new cases and 350,000 deaths reported in 2022, most of which occurred in low- and middle-income countries (World Health Organization, 2025). In Indonesia, cervical cancer remains a major public health concern, with high incidence and mortality that are associated with limited early detection, restricted access to healthcare technologies, and inadequate public awareness (Ferlay et al., 2021).

The main therapeutic approaches for cancer include surgery, radiotherapy, and chemotherapy (Ismail et al., 2021). Cisplatin is widely used as a first-line chemotherapeutic agent for several malignancies, including cervical cancer, because of its ability to induce DNA damage and cancer cell death (Ranasinghe et al., 2022). However, its clinical use is limited by dose-dependent toxicity, including nephrotoxicity and neurotoxicity, as well as the development of drug resistance (Elmorsy et al., 2024; Fang et al., 2021). These limitations have encouraged the investigation of combination strategies that may enhance cytotoxic efficacy while allowing dose reduction. Natural bioactive compounds have been explored as potential adjuvants to cisplatin-based chemotherapy because they may modulate cancer-related pathways and improve treatment response in experimental models (Dasari et al., 2022; Okem et al., 2023).

Eclipta alba Hassk. is a medicinal plant containing bioactive constituents such as wedelolactone, eclalbasaponin, luteolin, chlorogenic acid, and apigenin (Thenmozhi & Jayanthi, 2019; Myo et al., 2024). These compounds have been associated with anticancer-related mechanisms, including apoptosis induction, inhibition of cell proliferation, modulation of mitochondrial function, and antioxidant activity (Nelson et al., 2020; Yadav et al., 2017; Yang et al., 2023). Previous studies have reported that *E. alba* extracts can inhibit cancer cell proliferation, promote apoptosis, and reduce drug resistance-related mechanisms in experimental models (Dasari et al., 2022; Nelson et al., 2020). Other findings have also shown cytotoxic activity of *E. alba* against HeLa cervical cancer and HCT116 colon cancer cells (Subbulakshmi et al., 2025). However, direct evidence regarding the cytotoxic interaction between *E. alba* extract and cisplatin in cervical cancer cells remains limited.

Therefore, this study aimed to evaluate the cytotoxic interaction between cisplatin and *E. alba* extract in HeLa cervical cancer cells. The extract was characterized using thin-layer chromatography and LC–MS/MS, while cytotoxic activity was assessed using the MTT assay. The potential interaction between cisplatin and *E. alba* extract was further evaluated using combination index analysis to determine whether the fixed-dose combination produced synergistic, additive, or antagonistic effects.

2. METHODS

2.1. Materials and Instruments

The main instruments used in this study included a biosafety cabinet (BIOBASE), thin-layer chromatography chamber, ELISA microplate reader (Bio-Rad), freeze dryer (Lyovapor®), CO_2 incubator (BIOBASE), micropipettes (DragonLAB), oven (Memmert), rotary evaporator (IKA Werke RV 06 ML), UV cabinet at 254 and 366 nm, and vortex mixer (BIONEX). LC–MS/MS analysis was performed using an ACQUITY UPLC® H-Class system coupled with a Xevo G2-S QTof mass spectrometer.

The materials used included acetonitrile, aluminum chloride, formic acid, cisplatin, dimethyl sulfoxide, 96% ethanol, fetal bovine serum, ethanolic potassium hydroxide, RPMI-1640 medium, MTT reagent, penicillin–streptomycin, HeLa cervical cancer cells, TLC silica gel 60 F_{254} plates, 0.25% trypsin–EDTA, and vanillin–sulfuric acid reagent.

2.2. Plant Material and Extraction

Fresh leaves of *Eclipta alba* Hassk. were collected from Gowa, South Sulawesi, Indonesia, in July 2025. The plant material was authenticated by Dr. Abdul Rahim, S.Si., M.Si., Ph.D., Apt., Head of the Pharmacognosy Laboratory, Faculty of Pharmacy, Universitas Hasanuddin, Indonesia. An authenticated reference specimen is maintained at the Pharmacognosy Laboratory, Faculty of Pharmacy, Universitas Hasanuddin, under document number 035/SKIT/Farmakognosi/IX/2025, dated 24 September 2025. The taxonomic name was verified according to World Flora Online, WFO ID: wfo-0000001005.

The leaves were washed under running water, air-dried at room temperature until the moisture content was reduced, and ground into fine powder. A total of 170 g of dried *E. alba* leaf powder was extracted by maceration using 96% ethanol with a sample-to-solvent ratio of 1:10 (w/v) at room temperature. The maceration process was carried out in three cycles over 3 days. The obtained macerate was filtered and concentrated under reduced pressure using a rotary evaporator at 50°C, followed by freeze-drying for 48 h. The extraction yielded 17.38 g of crude extract,

corresponding to a yield of 10.22%. The extraction yield was calculated using the following formula:

$$Yield\ (\%) = \frac{weight\ of\ crude\ extract}{weight\ of\ dried\ plant\ powder} \times 100$$

The dried extract was stored in a desiccator until further analysis.

2.3. Phytochemical Screening by Thin-Layer Chromatography

Preliminary phytochemical screening was performed by thin-layer chromatography. A total of 0.5 mg of *E. alba* extract was dissolved in 2 mL of ethanol and mixed using a vortex mixer. The solution was spotted onto silica gel 60 F_{254} TLC plates and developed using toluene:chloroform:methanol, 5:4:1 v/v/v, as the mobile phase. After development, the plates were air-dried and sprayed with 5% aluminum chloride reagent for flavonoid detection, 5% ethanolic potassium hydroxide for phenolics and coumarins, and vanillin–sulfuric acid reagent for terpenoids and steroids. The plates were visualized under visible light and UV light at 254 and 366 nm, and retention factor values were calculated.

2.4. Compound Profiling by Liquid Chromatography–Tandem Mass Spectrometry

The extract was analyzed using liquid chromatography–tandem mass spectrometry. Samples were prepared by solid-phase extraction, eluted with methanol, evaporated to dryness, and reconstituted prior to analysis. Chromatographic separation was performed using an ACQUITY UPLC® H-Class system coupled to a Xevo G2-S QTof mass spectrometer, equipped with an ACQUITY UPLC® HSS C18 column, 1.8 µm, 2.1 × 100 mm, maintained at 50°C. The mobile phase consisted of water containing 5 mM ammonium formate as solvent A and acetonitrile containing 0.05% formic acid as solvent B. The flow rate was 0.2 mL/min, and the injection volume was 5 µL. The gradient program was 5% B from 0 to 2 min, increased to 95% B from 2 to 15 min, held at 95% B until 18 min, and returned to the initial condition for re-equilibration until 23 min.

Mass spectrometric detection was performed using electrospray ionization in positive mode over an m/z range of 50–1200. MS/MS data were acquired in MSE mode using MassLynx software. Fragment ions were associated with precursor ions based on retention-time alignment and accurate mass tolerance of ≤5 ppm. Compounds were tentatively annotated based on accurate mass measurements, mass error <5 ppm, observed adduct ions, predicted molecular formulae, and MS/MS spectral matching against the MassBank database within a 5 ppm mass tolerance. Only compounds meeting the predefined spectral similarity criteria were reported as tentative annotations.

2.5. Cell Culture

HeLa cervical cancer cells were obtained from the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Indonesia. Cells were maintained as monolayers in RPMI-1640 medium supplemented with 10% fetal bovine serum and 100 U/mL penicillin–streptomycin. Cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2. Once the cultures reached approximately 70% confluence, the cells were harvested using 0.25% trypsin–EDTA for subsequent experiments.

2.6. Cytotoxicity Assay

Cell viability was evaluated using the MTT assay. HeLa cells were seeded into 96-well flat-bottom plates at a density of 5×10^3 cells/well in 100 µL of complete RPMI-1640 medium and allowed to attach for 24 h at 37°C in a humidified 5% CO_2 incubator. After attachment, cells were treated with cisplatin alone at concentrations of 0.25, 0.5, 1, 2, and 4 µg/mL or with *E. alba* extract alone at concentrations of 62.5, 125, 250, 500, and 1000 µg/mL. For the combination treatment, a fixed sub-IC_{50} concentration of cisplatin, 0.42 µg/mL, was combined with the same concentration series of *E. alba* extract, 62.5–1000 µg/mL.

Cisplatin stock solution was prepared in DMSO and diluted in complete culture medium to obtain the final treatment concentrations. The final DMSO concentration did not exceed 0.1% v/v in any well. Vehicle control wells containing equivalent DMSO concentrations were included. Each treatment condition was tested in triplicate technical replicates, and the entire experiment was independently repeated three times as biological replicates.

Cells were incubated with the treatments for 24 h. After incubation, 50 µL of MTT solution, 5 mg/mL in phosphate-buffered saline, was added to each well and incubated for 4 h at 37°C. The medium was carefully removed, and 200 µL of DMSO was added to dissolve the formazan crystals. Plates were gently shaken for 10 min, and absorbance was measured at 550 nm using a microplate reader. Blank wells containing medium and MTT without cells were used for background subtraction. Cell viability was calculated relative to the untreated control using the following formula:

$$Cell\ viability\ (\%) = \frac{Absorbance\ of\ treated\ cells - Absorbance\ of\ blank}{Absorbance\ of\ untreated\ cells - Absorbance\ of\ blank} \times 100$$

IC_{50} values were determined using nonlinear regression analysis with a four-parameter logistic model in GraphPad Prism.

2.7. Combination Index Analysis

The combination index was calculated using CompuSyn software based on the Chou–Talalay method across the observed fraction affected values. The analysis was performed using a fixed sub-IC_{50} concentration of cisplatin combined with increasing concentrations of *E. alba* extract. Combination effects were interpreted according to CI values, where CI < 1 indicates synergism, CI = 1 indicates an additive effect, and CI > 1 indicates antagonism. Because the combination design used a fixed cisplatin concentration rather than a full dose–matrix design, the

interaction was interpreted as preliminary synergy under fixed-dose conditions.

2.8. Ethics Statement

This study used an established human cancer cell line and did not involve direct recruitment of human participants or use of experimental animals. The study protocol was reviewed and approved by the Research Ethics Committee of Sekolah Tinggi Ilmu Farmasi Makassar, approval number 07.150/KOMETIK/STIFA/VII/2025.

2.9. Statistical Analysis

Data analysis was performed using SPSS version 26, GraphPad Prism version 10.6.0, and CompuSyn. IC_{50} values were determined by nonlinear regression using a four-parameter logistic model in GraphPad Prism, with 95% confidence intervals and goodness-of-fit statistics. Data were expressed as mean ± standard deviation. Normality and homogeneity of variance were assessed using the Shapiro–Wilk test and Levene's test, respectively. Group comparisons were performed using one-way analysis of variance followed by Tukey's multiple comparison test. Statistical significance was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Extraction Yield and TLC-Based Phytochemical Screening

Ethanolic extraction of *E. alba* leaves produced 17.38 g of crude extract, corresponding to a yield of 10.22%. This result indicates that 96% ethanol extracted a measurable amount of polar to semi-polar constituents from the plant material. However, extraction yield alone does not determine biological activity; therefore, phytochemical screening and cytotoxicity testing were conducted to further characterize the extract.

Preliminary thin-layer chromatographic screening indicated the presence of flavonoids, coumarins, and terpenoid-related compounds in the ethanolic extract of *E. alba* (**Table 1**). Aluminum chloride produced yellow to yellowish-brown fluorescence, suggesting the presence of flavonoids. Potassium hydroxide visualization produced a blue fluorescent spot under UV 366 nm, indicating coumarin-type compounds, whereas vanillin–sulfuric acid produced purple spots, suggesting terpenoid or triterpenoid saponin constituents (Harborne et al., 1987; Febriani & Anam, 2022; Hadi et al., 2023). These results support the presence of secondary metabolites that may contribute to the cytotoxic effects observed in subsequent assays.

3.2. LC–MS/MS Profile of *E. alba* Extract

LC–MS/MS analysis was performed to support tentative identification of major constituents in the ethanolic extract. The chromatogram profile is shown in **Figure 1**, while the tentatively annotated compounds are summarized in **Table 2**. Five compounds were detected and tentatively identified based on retention time, mass-to-charge ratio, adduct ions, fragment ions, molecular formulae, and spectral matching: eclalbasaponin I, wedelolactone, pinoquercetin, luteolin, and quercitrin.

Table 1. TLC-Based Phytochemical Identification of *E. alba* Extract

Reagent	Rf	Visible color	UV 254 nm	UV 366 nm	Indicative compound class
$AlCl_3$	0.36	Pale yellow	Yellow	Yellowish brown	Flavonoids
Vanillin–sulfuric acid	0.25	Pale purple	Purple	Purple	Terpenoids/triterpenoid saponins
KOH	0.08	Faded green	Yellowish green	Blue	Coumarins/phenolics

Note: TLC screening provides preliminary phytochemical indication and does not confirm the identity of individual compounds.

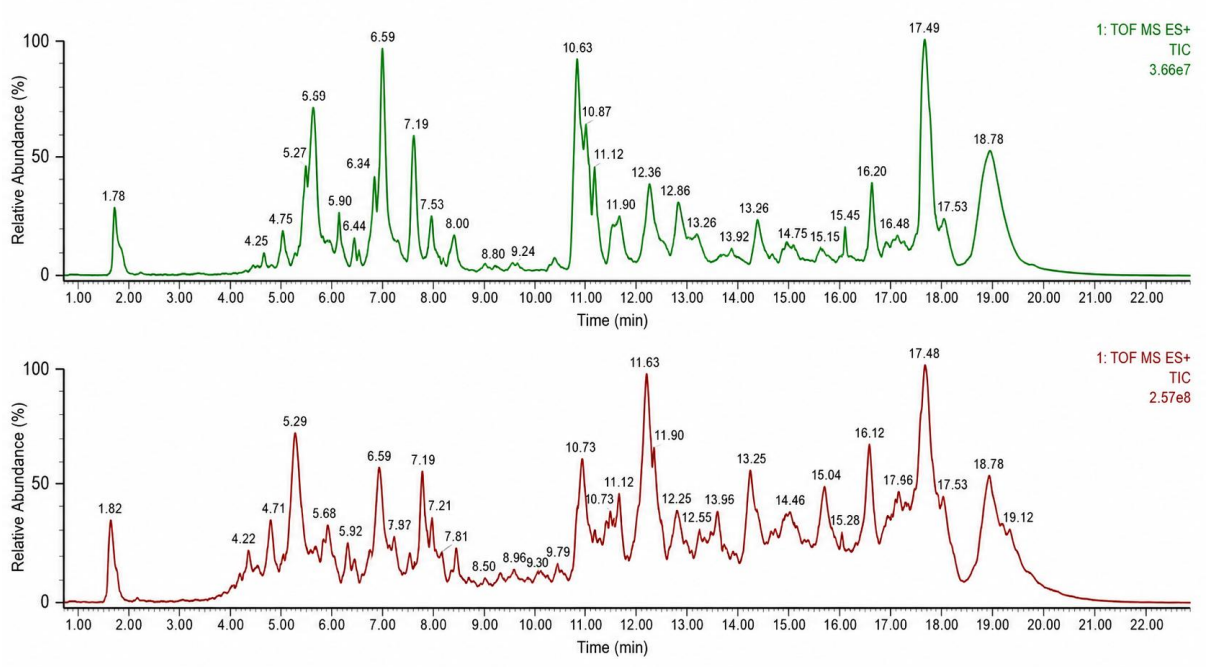

Figure 1. LC–MS/MS chromatogram profile of *E. alba* extract.

Table 2. LC–MS/MS Analysis of *E. alba* Leaf Extract

No.	Tentative identification	Retention time	m/z	Adduct ion	Fragment ions	Abundance	Molecular formula
1	Eclalbasaponin I	17.49	797.4664	$[M+H]^+$	184.0752; 335.2603; 613.4856; 595.4747	946,000	$C_{42}H_{68}O_{14}$
2	Wedelolactone	7.03	315.0512	$[M+H]^+$	287.0583; 269.0478; 200.0479; 167.0358; 161.0251; 155.0714	906,000	$C_{16}H_{10}O_7$
3	Pinoquercetin	5.50	317.0669	$[M+H]^+$	288.0628; 287.0550; 286.0472; 179.0339; 151.0389	747,000	$C_{16}H_{12}O_7$
4	Luteolin	6.79	287.0565	$[M+H]^+$	269.0478; 167.0356	461,000	$C_{15}H_{10}O_6$
5	Quercitrin	4.53	450.1160	$[M+H]^+$	301.0343; 302.0421; 147.0652; 103.0390; 89.0233	183,000	$C_{21}H_{20}O_{11}$

Note: Compound annotation was tentative and based on LC–MS/MS data, accurate mass, fragmentation pattern, and database comparison. Confirmation using authentic standards would be required for definitive identification.

The LC–MS/MS results are consistent with previous reports showing that *E. alba* contains wedelolactone, luteolin, and eclalbasaponin-related compounds (Du et al., 2018; Han et al., 2015). Among the tentatively identified compounds, eclalbasaponin I, wedelolactone, and pinoquercetin showed the highest relative abundances. Wedelolactone has been reported to exhibit anticancer, anti-inflammatory, antimicrobial, antidiabetic, and tissue-protective properties (Cai et al., 2025). Eclalbasaponins are triterpenoid saponins associated with antioxidant and anticancer activity, whereas luteolin and related flavonoids have been linked to antiproliferative, pro-apoptotic, and anti-inflammatory effects (Nelson et al., 2020; Zhu et al., 2025). These phytochemical findings provide a chemical basis for evaluating the cytotoxic activity of the extract.

3.3. Cytotoxic Effects of Cisplatin, *E. alba* Extract, and Their Combination

The MTT assay showed that cisplatin reduced HeLa cell viability in a concentration-dependent manner (**Figure 2A**). The reduction in cell viability was statistically significant compared with the untreated control group ($p < 0.0001$). Nonlinear regression analysis produced an IC_{50} value of 1.15 ± 0.16 µg/mL for cisplatin (Figure 3A), confirming its strong cytotoxic activity against HeLa cervical cancer cells. This result is consistent with the established mechanism of cisplatin, which induces DNA crosslinking, activates apoptosis-related pathways, and increases oxidative stress in cancer cells (Minerva et al., 2023; Ranasinghe et al., 2022).

The *E. alba* extract also reduced HeLa cell viability in a concentration-dependent manner (**Figure 2B**). The calculated IC_{50} value was 598.29 ± 42.89 µg/mL (Figure 3B), indicating that the extract showed measurable but weaker cytotoxic activity than cisplatin. This activity may be related to the presence of wedelolactone, eclalbasaponins, luteolin, and flavonoid-related compounds detected in the extract. Previous studies have reported that *E. alba* and its bioactive constituents can inhibit cancer cell proliferation, induce apoptosis, and modulate cancer-related signaling pathways (Nelson et al., 2020; Raina et al., 2021; Yadav et al., 2017; Yang et al., 2023). Therefore, the observed cytotoxicity is biologically plausible, although further mechanistic assays are required to confirm the underlying pathways.

The combination treatment produced a marked reduction in the IC_{50} value of *E. alba* extract under fixed sub-IC_{50} cisplatin conditions. When 0.42 µg/mL cisplatin was combined with increasing concentrations of *E. alba* extract, the extract IC_{50} decreased from 598.29 ± 42.89 µg/mL to 64.97 ± 5.41 µg/mL (**Figures 2C** and **3C**). This represents approximately a nine-fold reduction in the extract concentration required to inhibit 50% of cell viability. However, cisplatin remained the most intrinsically potent single agent. Thus, the combination result should be interpreted as a potential dose-sparing interaction under fixed-dose conditions, rather than evidence that the combination is intrinsically more potent than cisplatin alone.

Combination Index Analysis and Interpretation of Synergy

Combination index analysis was performed to evaluate whether the cytotoxic interaction between cisplatin and *E. alba* extract was synergistic, additive, or antagonistic. The median-effect parameters are presented in **Table 3**. The correlation coefficient values indicate acceptable fitting of the data to the median-effect equation, although the combination group showed a lower *r* value than the single-treatment groups. This suggests that the combination response should be interpreted cautiously.

The combination of cisplatin and *E. alba* extract produced CI values below 1 across the evaluated effect levels, with values of 0.25979, 0.18684, 0.13478, and 0.10812 at ED50, ED75, ED90, and ED95, respectively (**Table 4**). These values indicate a synergistic interaction under the fixed sub-IC_{50} cisplatin condition used in this study. However, because the experiment did not use a full dose–matrix or constant-ratio design, the results should be interpreted as preliminary evidence of synergy under the specific fixed-dose condition tested.

The observed synergy may be associated with complementary cytotoxic mechanisms between cisplatin and bioactive constituents of *E. alba*. Cisplatin is known to induce cytotoxicity through DNA crosslink formation, activation of DNA damage responses, mitochondrial oxidative stress, and apoptosis (Minerva et al., 2023; Ranasinghe et al., 2022). Meanwhile, compounds reported in *E. alba*, including wedelolactone and luteolin-related flavonoids, have been associated with modulation of PI3K/AKT,

RAS–RAF/MAPK, and apoptosis-related pathways in cancer models (Raina et al., 2021; Zhu et al., 2024). However, these mechanisms were not directly evaluated in the present study. Because this study measured only cell viability using the MTT assay, further apoptosis, ROS, and pathway-specific assays are needed to clarify the mechanism underlying the observed cytotoxic interaction.

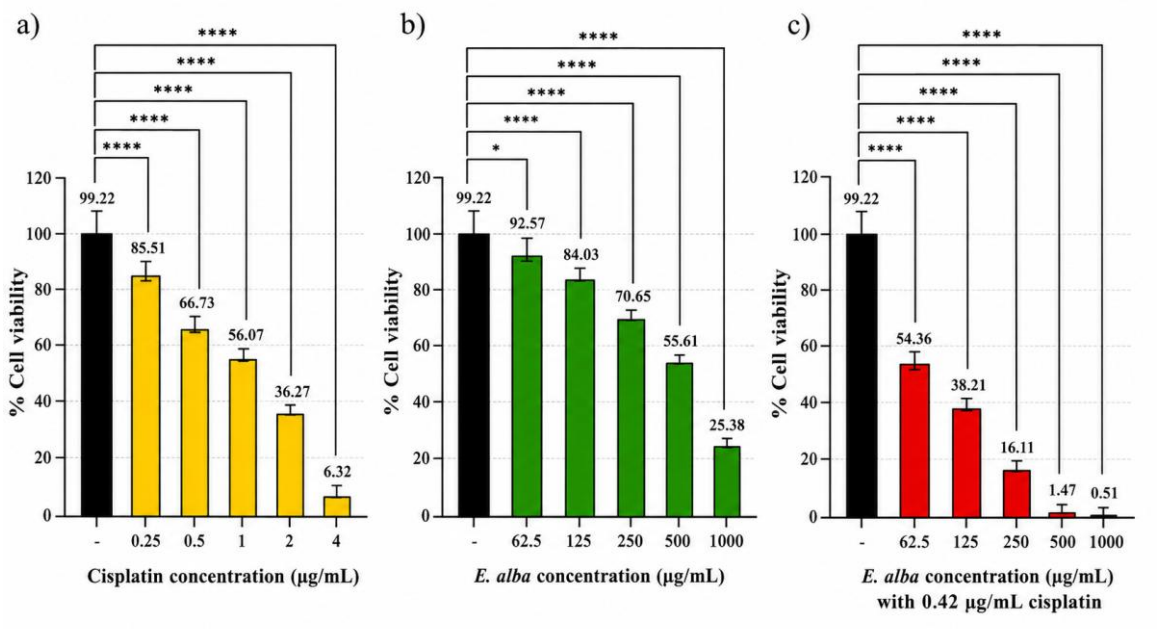

Figure 2. Effect of cisplatin, *E. alba* extract, and their combination on HeLa cervical cancer cell viability determined using the MTT assay. (A) Cisplatin; (B) *E. alba* extract; (C) fixed sub-IC_{50} cisplatin combined with *E. alba* extract. Data are presented as mean ± SD. **** $p < 0.0001$ indicates a significant difference compared with the untreated control group.

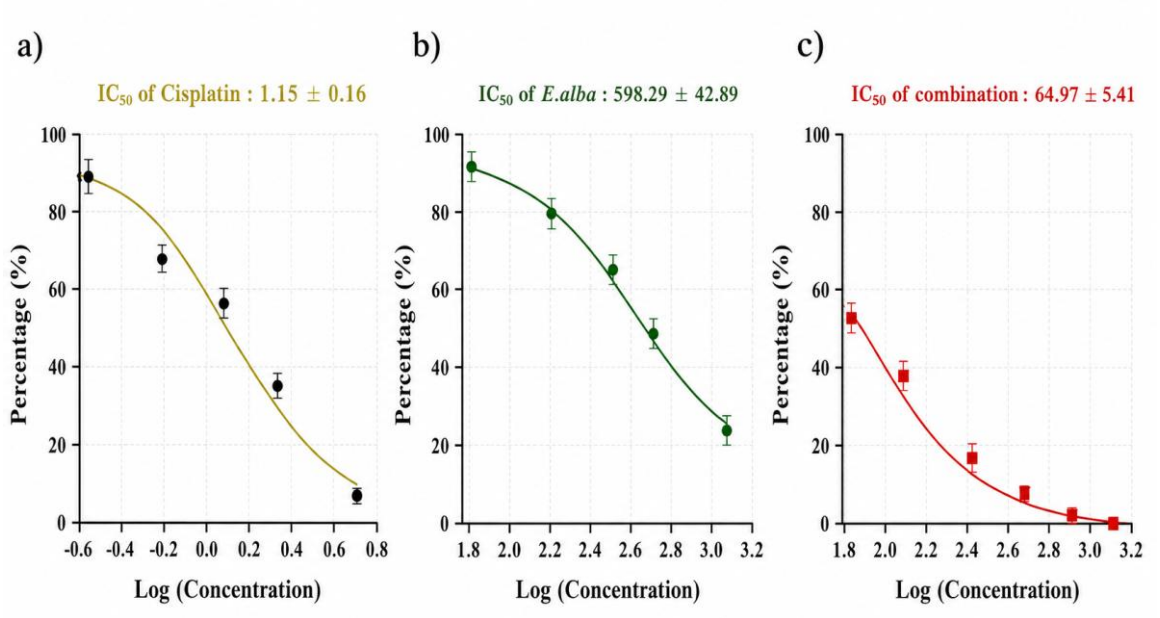

Figure 3. IC_{50} curves of cisplatin, *E. alba* extract, and their combination in HeLa cervical cancer cells. (A) Cisplatin; (B) *E. alba* extract; (C) fixed sub-IC_{50} cisplatin combined with *E. alba* extract.

Table 3. Median-Effect Parameters of Cisplatin, *E. alba* Extract, and Their Combination

Treatment	Dm (IC_{50})	m	r
E. alba	491.234	1.24604	0.99311
Cisplatin	0.92866	1.47189	0.96305
Combination	104.465	2.08831	0.92699

Note: Dm represents the median-effect dose required to produce 50% inhibition; m represents the slope of the median-effect plot; r represents the linear correlation coefficient indicating goodness of fit.

Table 4. Combination Index Values of Cisplatin and *E. alba* Extract at Different Effect Levels

Effect level	Fraction affected (Fa)	Combination index value	Interaction pattern
ED50	0.50	0.25979	Synergistic interaction
ED75	0.75	0.18684	Synergistic interaction
ED90	0.90	0.13478	Synergistic interaction
ED95	0.95	0.10812	Synergistic interaction

Note: CI values were calculated using the Chou–Talalay method with CompuSyn software. CI $<$ 1 indicates synergism, CI $=$ 1 indicates an additive effect, and CI $>$ 1 indicates antagonism.

Overall, the results suggest that *E. alba* extract may enhance the cytotoxic response of HeLa cells to cisplatin under fixed sub-IC_{50} conditions. This finding supports the potential of *E. alba* extract as a candidate adjuvant for further anticancer investigation. However, the study has several limitations. The combination design did not use a full dose–matrix approach, the mechanism of cell death was not directly assessed, and cytotoxic selectivity toward normal cells was not evaluated. In addition, phytochemical variability related to plant origin, harvesting conditions, and extraction procedures may affect reproducibility. Further studies using standardized extracts, apoptosis assays, ROS measurement, pathway analysis, normal cell models, full dose–matrix synergy testing, and in vivo validation are required before therapeutic implications can be established.

4. CONCLUSION

The combination of cisplatin and *Eclipta alba* extract increased cytotoxic activity against HeLa cervical cancer cells under fixed sub-IC_{50} cisplatin conditions. Cisplatin showed stronger single-agent cytotoxicity than the extract, while the combination reduced the IC_{50} value of the extract from 598.29 ± 42.89 µg/mL to 64.97 ± 5.41 µg/mL. Combination index values below 1 indicated a synergistic interaction within the tested fixed-dose design. These findings suggest that *E. alba* extract may enhance the in vitro cytotoxic effect of cisplatin under the experimental conditions used. Further studies using full dose–matrix testing, normal cell models, mechanistic assays, and in vivo evaluation are needed to confirm the relevance of this interaction.

AUTHOR CONTRIBUTIONS

Conceptualization, P.A.A., F.U., and Z.; methodology, P.A.A., S.S., M.I.H., N.A.Z.A., and W.H.; validation, F.U., Z., and L.; formal analysis, P.A.A., S.S., M.I.H., and N.A.Z.A.; investigation, P.A.A., S.S., M.I.H., W.H., N., and A.U.M.; resources, F.U., Z., and L.; data curation, P.A.A., S.S; writing—original draft preparation, P.A.A., S.S., and M.I.H.; writing—review and editing, F.U., Z., W.H., N., A.U.M., and L.; visualization, P.A.A. and N.A.Z.A.; supervision, F.U., Z., and L.; project administration, F.U.; funding acquisition, F.U. All authors have read and agreed to the published version of the manuscript.

INSTITUTIONAL REVIEW BOARD STATEMENT

The study protocol was reviewed and approved by the Research Ethics Committee of Sekolah Tinggi Ilmu Farmasi Makassar, approval number 07.150/KOMETIK/STIFA/VII/2025. The study used an established human cancer cell line and did not involve direct recruitment of human participants or experimental animals.

INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ROLE OF FUNDERS

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE (AI) USE

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to assist in improving the clarity, structure, grammar, and readability of the text. After using this tool, the authors thoroughly reviewed, edited, and verified the entire content to ensure that it accurately represents their own ideas, data, analyses, and interpretations. The authors take full responsibility for the integrity, accuracy, and originality of the published work.

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