



CYTOTOXIC ACTIVITY OF MELALEUCA SP. AND EUCALYPTUS SP. ESSENTIAL OILS AGAINST BREAST CANCER MCF-7 CELLS AND THEIR POTENTIAL ROLE IN CHEMOTHERAPY

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Abstract

Breast cancer remained one of the leading causes of cancer-related deaths among women, motivating the search for effective and accessible therapeutic support. Essential oils from *Melaleuca sp.* and *Eucalyptus sp.* had been reported to contain bioactive compounds with anticancer potential. This study aimed to compare their antiproliferative effects when combined with doxorubicin on MCF-7 breast cancer cells in vitro. MCF-7 cells were seeded in 96-well plates and treated with increasing concentrations of each essential oil together with doxorubicin for 48 hours. Cell viability was assessed using the resazurin assay. Both treatments reduced cell viability in a concentration-dependent manner. The combination of *Melaleuca sp.* and doxorubicin showed stronger inhibition, with an IC_{50} of 0.1576 $\mu\text{g/mL}$, while *Eucalyptus sp.* and doxorubicin produced an IC_{50} of 0.7792 $\mu\text{g/mL}$. These findings suggested that *Melaleuca sp.* demonstrated higher potency under the tested conditions. However, the absence of a doxorubicin-only control limited the interpretation of whether the effects were synergistic or simply additive. In conclusion, *Melaleuca sp.* appeared to have greater potential as a supportive natural agent in breast cancer treatment. Further studies were recommended to include full drug controls and mechanistic assays to better clarify the interaction with chemotherapy.

Keywords: Breast Cancer ; Essential Oil ; Eucalyptus Sp ; Melaleuca Sp ; MCF-7.

1. Introduction

The demand for natural substances with potential as bioactive agents in healthcare keeps growing, especially with the rising prevalence of chronic diseases, including cancer. Among various types of cancer, breast cancer ranks as the leading cause of death in women, both globally and nationally. This situation has encouraged many researchers to keep looking for alternative therapies that are not only effective, but also safer and more accessible to the public (Huang et al., 2022a; Lashen et al., 2021).

Standard treatment for breast cancer includes surgery, radiotherapy, and systemic pharmacotherapy, with chemotherapy remaining a cornerstone particularly in advanced and metastatic disease. Doxorubicin

is still widely used because of its broad antitumor activity and its ability to induce DNA intercalation, topoisomerase II inhibition, oxidative stress, and

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multiple forms of programmed cell death. However, its clinical use is limited by dose-dependent cardiotoxicity and other organ toxicities that continue to be actively investigated in recent studies. Platinum-based agents such as cisplatin also remain important in oncology, yet their clinical utility is constrained by toxicity and DNA-damaging effects that overlap with their antitumor activity (Dasari & Bernard Tchounwou, 2014; Kciuk et al., 2023).

The challenge of chemotherapy-related toxicity, combined with the growing problem of drug resistance, has driven interest in natural compounds as potential adjuncts to conventional cancer therapy. Essential oils (EOs) derived from medicinal plants have attracted particular attention due to their diverse bioactive compositions, multi-target mechanisms of action, and relatively low cytotoxicity toward normal cells. Combining natural bioactive agents with established chemotherapy drugs such as doxorubicin represents a promising strategy to improve therapeutic outcomes in breast cancer (Thalappil et al., 2024).

Melaleuca sp. and Eucalyptus sp. are plants native to Australia that have been widely cultivated in Indonesia, including in West Java, for essential oil production. These plants have long been used in traditional medicine, especially for respiratory problems and infections (Borotová et al., 2022; Joy et al., 2020). The essential oils of Eucalyptus sp. and Melaleuca sp. contain active compounds like 1,8-cineole and other bioactive substances that have been reported to show antibacterial (Borotová et al., 2022; Čmíková et al., 2023; Merghni et al., 2023; Putri et al., 2023), antiviral (Markowski & Junka, 2025), antioxidant (Borotová et al., 2022; Khan, 2025), and anti-inflammatory properties (Hoch et al., 2023). Interestingly, recent studies have shown that these same compounds may also have biological effects on cancer cells. Previous research has demonstrated that the lipophilic fraction of *Eucalyptus camaldulensis* significantly reduces the viability of MCF-7 cells (Huang et al., 2022). Given that multiple *Eucalyptus* species share similar bioactive constituents particularly 1,8-cineole, comparable biological activities have been reported across the genus (Shiekh et al., 2025). In addition, *Melaleuca* sp. has been reported to cause antitumor effects in vitro on MCF-7 and 4T1 cells through mechanisms that trigger apoptosis and inhibit cancer cell proliferation (Assmann et al., 2018).

In the present study, the Eucalyptus essential oil was obtained from an unidentified Eucalyptus sp. cultivated at BPTROA Lembang, West Java, whereas the Melaleuca essential oil was sourced from the Pabrik Minyak Kayu Putih (PMKP) in Jatimunggul, Indramayu. Despite the growing body of evidence supporting the anticancer potential of Eucalyptus sp. and Melaleuca sp. essential oils, comparative studies evaluating their combined effects with doxorubicin on breast cancer cell viability remain limited, particularly among locally available Indonesian plant sources. Therefore, this study aims to evaluate and compare the in vitro antiproliferative activity of Eucalyptus sp. and Melaleuca sp. essential oils when combined with doxorubicin on MCF-7 human breast cancer cells, using the resazurin cell viability assay. Cisplatin was included as the positive control to provide a standardized cytotoxic reference. The findings are intended to contribute foundational data toward the development of evidence-based, plant-derived adjunct therapies for breast cancer treatment.

2. Method

Media, Sample, and Control Preparation

Cells were cultured using complete RPMI medium containing 10% Fetal Bovine Serum (FBS) and antibiotics. Cisplatin was used as the positive control. The essential oils were dissolved using Dimethyl sulfoxide (DMSO) to prepare stock solutions at specific concentrations. The cell viability test reagent used was a working solution of Resazurin (Resazurin Sodium Salt).

Cell Culture and Preparation

The basal culture medium was prepared using Roswell Park Memorial Institute Medium (RPMI) supplemented with 10% Fetal Bovine Serum (FBS) and 50 µL/50mL antibiotics. Positive control was prepared using Cisplatin as an antiproliferative agent. Test samples were diluted from the highest concentration stock into a series of dilutions in media that showed no inherent toxicity to cells. Cells were cultured to a minimum confluency of 70%, then washed twice with 1 mL phosphate buffered saline (PBS) before adding 1 mL Trypsin-EDTA solution and incubating for 5 minutes at 37° C until cell dispersion

was achieved. Dispersed cells were centrifuged at 3000 rpm for 5 minutes, the supernatant was removed, and cells were resuspended in prepared culture medium. Cell viability was determined by trypan blue exclusion using a hemocytometer, and cells were resuspended to a final density of 170,000 cells/mL in culture medium.

Cell Seeding

Cell seeding was performed by adding 100 μ L of cell suspension into 96-well plates containing culture medium, achieving 17,000 cells per well. Plates were incubated for 24 hours at 37°C under 5% CO₂ to ensure cells reached minimum 70% confluency prior to treatment. Samples and positive control (Cisplatin) were added via micropipet as 100 μ L serial dilutions into corresponding wells, with negative controls containing media without samples or Cisplatin. Plates were re-incubated for 48 hours at 37°C under 5% CO₂.

Sample and Control Treatment

Eight serial two-fold dilutions of each essential oil were prepared from the stock solution using culture medium, yielding concentrations of 0.06, 0.12, 0.24, 0.49, 0.98, 1.95, 3.91, and 7.81 μ g/mL. Doxorubicin was prepared as a fixed sub-therapeutic concentration and combined with each dilution of the essential oil prior to treatment. The medium in the seeded 96-well plate was removed and replaced with 100 μ L of the appropriate treatment solution per well according to the following experimental groups: (1) Eucalyptus sp. essential oil + doxorubicin (eight concentration levels); (2) Melaleuca sp. essential oil + doxorubicin (eight concentration levels); (3) cisplatin as the positive control (43.18 μ M), included to provide a standardized cytotoxic reference; (4) solvent control (vehicle only, without essential oil or drug), to confirm the absence of solvent-induced cytotoxicity; and (5) media + cell control (untreated cells), representing 100% baseline cell viability. Each condition was tested in at least duplicate wells. Incubation was continued for 48 hours at 37°C in a 5% CO₂ atmosphere under standard culture conditions. Antiproliferative Assay and Absorbance Measurement Following the 48-hour incubation period, the antiproliferative assay working solution was prepared using Resazurin Sodium Salt-Powder from BioReagent. Culture medium was removed from each well, fresh media (9 mL) was added to a reagent tube, followed by addition of 1 mL Resazurin Sodium Salt-Powder solution (10 μ L reagent per 90 μ L media). One hundred microliters of the mixed solution was distributed into each microplate well and incubated for 1-2 hours until color change was observed (from blue to red fluorescence), indicating active metabolic activity in viable cells. Absorbance was measured at wavelength 570 nm (reference: 600 nm) using a Multimode Reader, with absorbance values increasing proportionally to the number of metabolically active cells capable of reducing resazurin to the fluorescent red resorufin. Absorbance data were analyzed to determine cell growth inhibition and calculate the IC₅₀ value of test samples.

3. Result and Discussion

Both combination treatments exhibited concentration-dependent antiproliferative activity against MCF-7 human breast cancer cells, as shown by the progressive reduction in cell viability across the tested concentration range (Tables 1 and Table 2). IC₅₀ is a widely used pharmacological parameter that reflects the concentration required to inhibit 50% of cell viability and is commonly applied to assess the cytotoxic potency of test compounds in vitro (Sánchez-Díez et al., 2025). A lower IC₅₀ value reflects greater antiproliferative potency, indicating that a smaller concentration is sufficient to inhibit 50% of cell growth (Berrouet et al., 2020). In this study, the IC₅₀ values were 0.1576 μ g/mL for the Melaleuca sp. + doxorubicin group and 0.7792 μ g/mL for the Eucalyptus sp. + doxorubicin group, indicating that the Melaleuca combination had greater antiproliferative potency under the tested conditions.

Cisplatin was included as a positive control to provide a standardized cytotoxic reference in the MCF-7 assay, since it is a well-established antineoplastic agent used in cytotoxicity studies (Ćwiklińska-Jurkowska et al., 2023; Dasari & Bernard Tchounwou, 2014; Kashkoulinejad-Kouhi et al., 2021; Mirmalek et al., 2016). In this study, cisplatin reduced MCF-7 cell viability to approximately 38.97% and 42.51% in the Eucalyptus and Melaleuca assay plates, respectively, confirming the responsiveness of the cell line and

the validity of the experimental system. Notably, while cisplatin served as the cytotoxicity reference control, doxorubicin is an anthracycline antibiotic that inhibits topoisomerase II and intercalates into DNA. It was selected as the chemotherapeutic partner for the essential oil combinations, given its established clinical role as a first-line agent in breast cancer treatment and its well-characterized activity against MCF-7 cells (Kciuk et al., 2023; Tacar et al., 2013). The concurrent use of two different chemotherapy agents in these respective roles is methodologically justified, as each serves a distinct experimental function: cisplatin as an assay validity benchmark, and doxorubicin as a clinically relevant combination partner.

Table 1. Cell viability percentage of MCF-7 cells. (*Eucalyptus sp.* + Doxorubicin)

Concentration ($\mu\text{g/mL}$)	% Cell Viability (mean $\pm$ SEM)
0,06	87,42 $\pm$ 0,28
0,12	81,19 $\pm$ 0,00
0,24	73,27 $\pm$ 0,96
0,49	48,80 $\pm$ 0,20
0,98	38,00 $\pm$ 0,00
1,95	30,07 $\pm$ 0,63
3,91	25,63 $\pm$ 0,00
7,81	0,00 $\pm$ 0,00

Control:

- Media + cell: 103,68 $\pm$ 0,28
- Cisplatin 43,18 μM : 38,97 $\pm$ 0,96
- Solvent: 100,00 $\pm$ 0,00

Table 2. Cell viability percentage of MCF-7 cells. (*Melaleuca sp.* + Doxorubicin)

Concentration ($\mu\text{g/mL}$)	% Cell Viability (mean $\pm$ SEM)
0,06	82,17 $\pm$ 0,92
0,12	64,35 $\pm$ 0,41
0,24	49,12 $\pm$ 0,00
0,49	43,92 $\pm$ 0,00
0,98	38,42 $\pm$ 0,20
1,95	34,80 $\pm$ 0,10
3,91	33,77 $\pm$ 0,00
7,81	0,00 $\pm$ 0,00

Control:

- Media + cell: 103,92 $\pm$ 0,28
- Cisplatin 43,18 μM : 42,51 $\pm$ 0,92
- Solvent: 100,00 $\pm$ 0,00

The findings from this study show that treatment with *Melaleuca* combined with doxorubicin appears to suppress MCF-7 cell growth more effectively than *Eucalyptus* combined with doxorubicin. The stronger activity observed in the *Melaleuca sp.* combination may be related to differences in the chemical composition of the essential oils, since essential oils have been reported to exert anticancer effects through apoptosis induction, oxidative stress modulation, membrane disruption, and interference with cell proliferation pathways (Blowman et al., 2018a; Sharma et al., 2022). In this context, the higher potency of the *Melaleuca* combination suggests that its constituents may have interacted more effectively with doxorubicin to suppress MCF-7 cell growth. However, because the present study did not include a doxorubicin-only group, the observed effect cannot yet be classified as synergistic or additive with certainty.

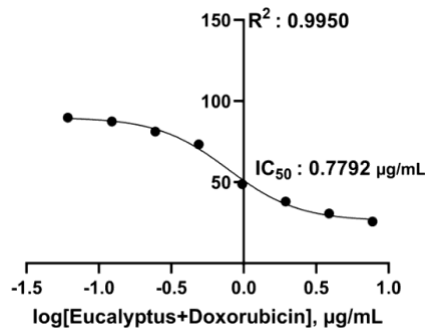


Figure 1. Dose-response curve (*Eucalyptus*+*Doxorubicin*)

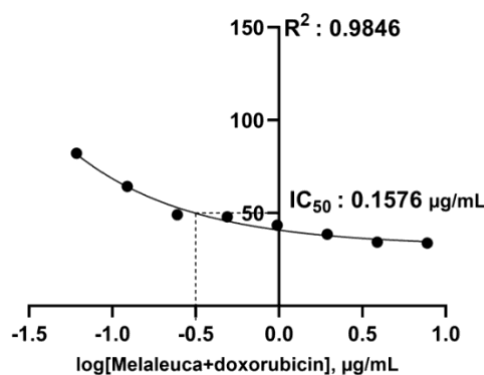


Figure 2. Dose-response curve (*Melaleuca*+*Doxorubicin*)

The graphs in Figure 1 and Figure 2 show a concentration-dependent decrease in MCF-7 cell viability for both samples. The curve for the Melaleuca + doxorubicin treatment shifts more to the left compared to Eucalyptus + doxorubicin, which suggests a stronger inhibitory effect at lower concentrations. The non-linear regression analysis resulted in IC₅₀ values of 0.1576 µg/mL for Melaleuca and 0.7792 µg/mL for Eucalyptus, with R² values close to 1. The leftward shift of the Melaleuca + doxorubicin curve indicates that this combination has a stronger inhibitory effect, meaning a lower dose is needed to achieve the same reduction in cell viability.

The superior antiproliferative potency of the Melaleuca sp. + doxorubicin combination is likely attributable to differences in the bioactive constituent profile of the two essential oils. Melaleuca alternifolia essential oil is characterised by high concentrations of terpinen-4-ol, a monoterpene alcohol that has been reported to induce apoptosis in several cancer cell lines through the mitochondrial intrinsic pathway, involving increased reactive oxygen species (ROS) generation, collapse of mitochondrial membrane potential, and activation of caspases 3 and 9 (Banjerdpongchai & Khaw-On, 2013; Blowman et al., 2018b; Lin et al., 2012; Sharma et al., 2022). Importantly, (Assmann et al., 2018) demonstrated that Melaleuca sp. concentrate selectively induced mitochondrial apoptosis in MCF-7 breast cancer cells while sparing non-cancerous cells, suggesting a degree of cancer cell selectivity that may complement the mechanism of doxorubicin. In contrast, Eucalyptus-derived extracts or essential oil fractions, which often contain 1,8-cineole as a major constituent (Čmíková et al., 2023), have demonstrated antiproliferative and pro-apoptotic properties in cancer cell models, including MCF-7, with mechanisms that may involve oxidative stress and cell cycle perturbation (Huang et al., 2022b; Zhou et al., 2016), but appears to exert comparatively lower cytotoxic potency against MCF-7 cells under the conditions tested in this study. These mechanistic differences in bioactive composition may explain why the Melaleuca sp. + doxorubicin combination required a five-fold lower concentration to achieve equivalent inhibition of MCF-7 cell growth compared to the Eucalyptus sp. + doxorubicin group. These findings are consistent with the

broader literature suggesting that terpinen-4-ol-rich essential oils, including those from *Melaleuca* sp, may serve as promising adjunct agents to conventional chemotherapy in breast cancer by simultaneously engaging multiple pro-apoptotic pathways (Assmann et al., 2018; Rodenak-Kladniew et al., 2020).

Although the absence of a doxorubicin-only control limits formal interpretation of synergy, the present findings still provide preliminary evidence that the essential oils may contribute to the antiproliferative response under co-treatment conditions. Future studies should include a doxorubicin-only control, mechanistic assays to clarify the underlying cell death pathways, and additional in vitro or in vivo models to confirm the therapeutic potential of these essential oils in breast cancer.

4. Conclusion and Suggestion

This study provides preliminary evidence that natural compounds from *Melaleuca* sp. and *Eucalyptus* sp. may reduce the viability of MCF-7 breast cancer cells when combined with doxorubicin. Among the two tested essential oils, *Melaleuca* sp. showed greater antiproliferative activity than *Eucalyptus* sp., as indicated by its effectiveness at lower concentrations. These findings suggest that locally available medicinal plants may have potential as supportive agents in breast cancer treatment and warrant further investigation.

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6. References

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