



Original Article

In Vitro Cytotoxic Activity of the Ethanolic Leaf Extract of *Myristica fragrans* Houtt. On MCF-7 Breast Cancer Cells

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Abstract

The high mortality rate due to breast cancer in women globally, including in Indonesia, has triggered the urgency of finding alternative therapies that are more affordable and have minimal side effects. This study aims to evaluate the cytotoxic effects of ethanolic leaf extract of nutmeg (*Myristica fragrans* Houtt) on the MCF-7 breast cancer cell line. The extraction process was carried out by maceration with 70% ethanol solvent, followed by cell viability testing using the PrestoBlue assay at various concentrations from 7.81 to 1000 µg/mL. In this experiment, cisplatin was applied as a positive control, while DMSO along with culture media acted as negative controls and untreated MCF-7 Cells. The test results showed that increasing the extract concentration caused a gradual decrease in MCF-7 cell viability, with the most obvious inhibitory effect seen in the morphology of cells exposed to concentrations of 250, 500 and 1000 µg/mL. The IC₅₀ value obtained was 415.80 µg/mL, so its cytotoxic activity was categorized as moderate cytotoxic.

Keywords: *Myristica fragrans*; Cytotoxicity; MCF-7; Breast cancer; Ethanolic extract.

INTRODUCTION

Breast cancer ranks first globally in terms of cancer prevalence and remains the leading cause of cancer-related mortality among women. This pathological condition is triggered by abnormalities in the mechanisms that regulate the cell cycle, resulting in uncontrolled cellular proliferation and abnormal cell division, which ultimately lead to the formation of tumors within breast tissue (Hanahan, 2022).

These cancer cells can spread (metastasize) to other parts of the body and may become fatal if not diagnosed and treated appropriately. According to World Health Organization (WHO) data, breast cancer was responsible for approximately 670,000 deaths worldwide in 2022. notably, the disease burden indicates that nearly half of all breast cancer cases occur in women

without any identifiable specific risk factors. Breast cancer is the most commonly diagnosed malignancy among women globally, ranking as the leading cancer type in 157 out of 185 countries. Although it predominantly affects women, men can also develop breast cancer, accounting for approximately 0.5-1% of all cases (WHO, 2025).

In Indonesia, according to data released by the International Agency for Research on Cancer (IARC), the number of new breast cancer cases is estimated to reach 68,858 annually (GLOBOCAN, 2020). In modern oncology, one of the fundamental principles of cancer treatment is the implementation of therapeutic strategies that specifically target particular cancer cell types (Wang *et al.*, 2025). Breast cancer is also a highly heterogeneous disease that can arise from a

variety of distinct genetic alterations, resulting in diverse biological characteristics and clinical manifestations among patients (Lüönd *et al.*, 2021). In cell culture-based studies, the MCF-7 cell line is widely used as a research model due to its close resemblance to the luminal A phenotype, particularly its positive estrogen receptor (ER-positive) expression status, which represents the most prevalent subtype of breast cancer among women (Horwitz *et al.*, 1975).

Conventional treatment modalities, including chemotherapy, radiotherapy, and surgery, have demonstrated considerable efficacy; however, they are often associated with significant adverse effects and the potential development of drug resistance (Zafar *et al.*, 2025). In addition, the high cost of treatment and limited access to advanced therapies, particularly in rural areas, remain major challenges in the management of this disease. These limitations have driven efforts to develop and explore alternative therapeutic approaches that offer improved safety profiles, adequate therapeutic efficacy, and greater affordability, particularly through the utilization of bioactive compounds derived from natural products (Cragg and Newman, 2005).

Nutmeg (*Myristica fragrans* Houtt) is one of Indonesia's valuable biological resources, originating from the Maluku Islands, and has long been recognized as an important commodity in the global spice trade (Basir *et al.*, 2018). Historically, the Maluku Islands, particularly Banda Island, served as the world's sole source of nutmeg for centuries before its cultivation expanded to other tropical regions (Milton, 2019). All administrative regions within North Maluku Province are actively involved in nutmeg production, with a total

of 78,294 producing households recorded in the latest statistical report published by the North Maluku Central Statistics Agency (BPS) in 2025. In addition to its substantial economic value, nutmeg contains a variety of bioactive compounds that exhibit diverse pharmacological properties, including antifungal activity (Rajih *et al.*, 2023). Previous studies have also demonstrated that nutmeg fruit flesh possesses antidiabetic potential at a dose of 300 mg/kg body weight (Nur *et al.*, 2024). While nutmeg seeds have been reported to induce apoptosis in malignant melanoma (B16-F10) cells (Lesmana *et al.*, 2021).

Nevertheless, not all plants exhibit strong anticancer activity. Several studies investigating local plants from the Ternate region have reported relatively low inhibitory effects against the growth of MCF-7 breast cancer cells. For example, blue butterfly pea (*Clitoria ternatea*) flowers demonstrated an IC₅₀ value that falls within the non-toxic category (Alshaya *et al.*, 2022). Therefore, further scientific investigation is required to evaluate the anticancer potential of other plant parts that are suspected to possess cytotoxic properties, including (*Myristica fragrans* Houtt) leaves (Lesmana *et al.*, 2021).

The assessment of cytotoxic activity requires sensitive and reliable analytical methods, one of which is the PrestoBlue assay, which is commonly used to determine cell viability based on the metabolic activity of living cells. Compared with the MTT assay, the PrestoBlue method offers several advantages, including higher sensitivity, a more straightforward experimental procedure, and the elimination of the need for formazan crystal solubilization (Luzak *et al.*, 2022).

Based on the rationale presented in the background, this study aimed to assess the inhibitory effect of the ethanol extract of nutmeg leaves on the viability of MCF-7 breast cancer cells and to determine the 50% inhibitory concentration (IC₅₀) using the PrestoBlue assay.

METHODS

Equipment

Experimental procedures were performed under aseptic conditions in a Thermo Scientific 1300 Series A2 Biosafety Cabinet (BSC). Cell separation and an 8000DH series CO₂ incubator. Cell morphology was examined using an EVOS XL Core microscope, and absorbance measurements were obtained using a Tecan Infinite M200 PRO multimode microplate reader.

Materials

The study utilized 1.5 mL microtubes (Gene Follower MCTB015), 15 mL centrifuge tubes (Biologix 109151), 75 mL tissue culture flasks (NEST 708001), and 96-well microplates (NEST 701001). The reagents used included cisplatin (EDQM C2210000), antibiotics (Sigma-Aldrich P4333), dimethyl sulfoxide (DMSO; Merck D1435), phosphate-buffered saline (PBS; Gibco 18912-014), PrestoBlue™ Cell Viability Reagent (Thermo Fisher Scientific A13262), RPMI culture medium (Gibco 11875-093), fetal bovine serum (FBS; Gibco 10270-106), Trypsin-EDTA (Gibco 25200-056), and Trypan Blue solution (Sigma-Aldrich T-8154).

Experimental Procedures

Preparation of *Myristica fragrans* Leaf Ethanolic Extract

Fresh nutmeg leaves were collected from Ngade Village, Ternate, North

Maluku, Indonesia. The leaves were first washed under running water to remove impurities, then dried and ground into simplicia powder. Sample extraction was performed using the maceration method with 70% ethanol as the extraction solvent. The powdered crude plant material (simplicia) was placed in an extraction vessel and immersed in the solvent for 3 × 24 hours (72 hours), with stirring performed every 24 hours to ensure uniform solvent contact and facilitate the extraction of bioactive compounds. After maceration, the mixture was filtered using filter paper to obtain a liquid extract (filtrate). The filtrate was subsequently concentrated to obtain a viscous extract using a rotary evaporator operated at 80 rpm and a controlled temperature of 40°C.

Cytotoxicity Assay on MCF-7 Cells Using the PrestoBlue Assay

The MCF-7 breast cancer cell line used in this study was obtained from the American Type Culture Collection (ATCC) and maintained at the Central Laboratory of Universitas Padjadjaran, Bandung, Indonesia. Cells were cultured in RPMI (Roswell Park Memorial Institute) basal medium supplemented with 10% fetal bovine serum (FBS) as a nutrient source. To maintain culture sterility, streptomycin and penicillin were added at final concentrations of 100 U/mL each, with 50 µL of the antibiotic mixture added to every 50 mL of culture medium. Cisplatin was used as the positive control, while the negative controls included an untreated cell control, a solvent control, and a medium

blank. The untreated cell control consisted of cells cultured without extract treatment or added DMSO, whereas the solvent control comprised cells exposed to 2% DMSO in the absence of the extract. The medium blank contained culture medium and PrestoBlue™ reagent without cells.

The extract test solutions were prepared at concentrations of 7.81, 15.63, 31.25, 62.50, 125, 250, 500, and 1000 µg/mL, with each treatment performed in duplicate. Cell viability was evaluated using the PrestoBlue™ Cell Viability Reagent. Prior to treatment, cells were cultured until they reached at least 70% confluency. The cells were then incubated for 24 hours in a humidified incubator

containing 5% CO₂ at 37°C to ensure optimal cell growth.

Subsequently, 100 µL of each extract concentration and cisplatin (53 µM) as the positive control were added to individual wells of a 96-well microplate containing the cultured cells using a micropipette. The plate was then incubated for an additional 48 hours. Following incubation, the spent medium in each well was removed and replaced with 100 µL of a fresh medium–PrestoBlue™ mixture prepared at a 9:1 ratio (9 mL medium and 1 mL reagent). The cells were further incubated for 1–2 hours until a color change occurred, indicating cellular metabolic activity. Absorbance values were quantified by measuring dual wavelengths of 570 nm and 600 nm using a multimode microplate reader (Rampersad, 2012).

RESULTS

Table 1. Effect of Different Concentrations of *Myristica fragrans* Houtt. Leaf Ethanolic Extract on MCF-7 Cell Viability

	Medium	Medium + Cells	Cisplatin	Solvent	Sample Concentrations (µg/mL)							
					1000,00	500,00	250,00	125,00	62,50	31,25	15,63	7,81
Absorbance	0,4492	0,7193	0,5466	0,7231	0,4774	0,5626	0,6243	0,6888	0,7158	0,7261	0,7311	0,7213
	0,4529	0,7171	0,5530	0,7203	0,4761	0,5530	0,6213	0,6961	0,7343	0,7341	0,7385	0,7383
Cell Viability (%)		100,41	35,77	101,83	9,86	41,76	64,85	88,99	99,10	102,96	104,83	101,16
		99,59	38,16	100,79	9,38	38,16	63,73	91,73	106,03	105,95	107,60	107,52
Mean Cell Viability (%)		100,00	36,96	101,31	9,62	39,96	64,29	90,39	102,56	104,45	106,21	104,34
SEM		0,41	1,20	0,52	0,24	1,80	0,56	1,37	3,46	1,50	1,38	3,18
Normalized Cell Viability (%)		98,71	36,49	100,00	9,50	39,44	63,46	89,19	101,24	103,10	104,84	102,99

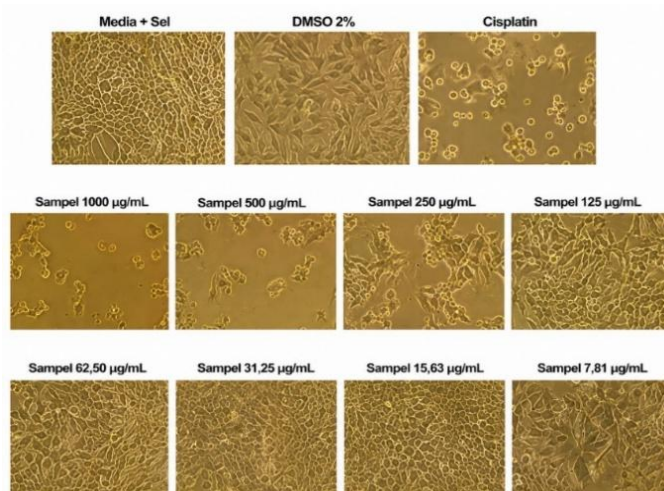


Figure 1. Morphological Observation of Control and *Myristica fragrans* Houtt Leaf Extract-Treated MCF-7 Cells

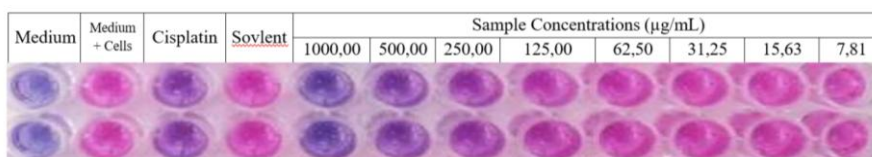


Figure 2. Photographic Documentation of Well Plates Containing MCF-7 Cells Treated with *Myristica fragrans* Houtt Leaf Ethanolic Extract

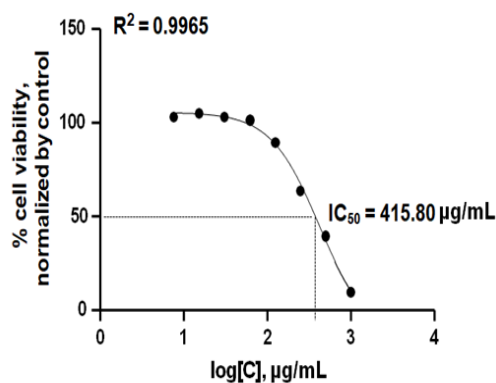


Figure 3. IC₅₀ of *Myristica fragrans* Houtt Leaf Ethanolic Extract on MCF-7 Breast Cancer Cells

DISCUSSION

The cytotoxic activity of the ethanolic extract of (*Myristica fragrans* Houtt) Leaves against MCF-7 breast cancer cells was initially evaluated at a

concentration of 1000 µg/mL using the PrestoBlue assay. This assay was conducted to assess the effectiveness of the extract in inhibiting cancer cell growth. The observed inhibitory activity is

associated with the cytotoxic properties of the extract, which can suppress cell proliferation and induce cancer cell death (Luzak *et al.*, 2022). The PrestoBlue assay employs resazurin as a cell viability indicator. In viable cells, resazurin is reduced to resorufin through metabolic activity involving coenzymes such as NADH, NADPH, FADH, FMNH, and cytochromes. This reaction is indicated by a color change of the solution from blue to light purplish-red or bright red, reflecting the presence of ongoing cellular metabolic activity (Vieira-da-Silva and Castanho, 2023).

Morphological observations and cell color changes presented in Figures 1 and 2 demonstrated clear differences among the treatment groups. The negative control groups (culture medium and 2% DMSO) exhibited high cell viability with normal cellular morphology, whereas the positive control treated with cisplatin showed a significant reduction in the number of viable cells. In cells treated with the ethanolic extract of *Myristica fragrans* Hoult leaves at concentrations ranging from 1000 to 7.81 $\mu\text{g/mL}$, a dose-dependent increase in cell death was observed. At higher extract concentrations (1000 and 250 $\mu\text{g/mL}$), morphological changes indicative of reduced cell viability was observed. Although some of these changes resembled those observed in the cisplatin-treated positive control, the cytotoxic effects of the extract were not necessarily equivalent to those of cisplatin. The number of viable cells was markedly reduced at higher extract concentrations. The number of viable cells was markedly reduced, as evidenced by the predominance of a bluish-purple coloration in the treated wells. This finding indicates a low level of resazurin

reduction to resorufin, suggesting that the majority of cells had undergone cell death. In contrast, lower extract concentrations resulted in a relatively higher number of viable cells. These findings indicate that the cytotoxic effect of the extract increased in a dose-dependent manner.

As a positive control, cisplatin is known to exert its anticancer effects through multiple molecular mechanisms, including the induction of oxidative stress, activation of the MAPK and JNK signalling pathways, modulation of AKT proteins, and DNA damage that triggers the activation of the p53 protein, ultimately leading to apoptosis of cancer cells (Cecati *et al.*, 2026). Cisplatin is capable of forming DNA crosslinks, particularly 1,2-intrastrand crosslinks, which play a critical role in inhibiting cancer cell replication and transcription. In contrast, its geometric isomer, transplatin, exhibits little to no significant anticancer activity. This difference is attributed to steric hindrance, which prevents transplatin from effectively forming DNA crosslinks and interacting with DNA in a biologically active manner (Komeda, 2019).

The study results revealed that the IC_{50} value of the ethanolic extract of *Myristica fragrans* Hoult leaves was 415.80 $\mu\text{g/mL}$, indicating moderate cytotoxic activity. This classification is based on the criterion that compounds exhibiting high inhibitory activity possess IC_{50} values below 100 $\mu\text{g/mL}$, whereas compounds with IC_{50} values ranging from 100 to 1000 $\mu\text{g/mL}$ are categorized as moderately cytotoxic. Compounds with IC_{50} values exceeding 1000 $\mu\text{g/mL}$ are considered non-cytotoxic (Arbiastutie *et al.*, 2022). Compounds with strong cytotoxic activity have the potential to be

developed as chemotherapeutic agents, while those exhibiting moderate cytotoxicity may be more suitable as chemo-preventive agents capable of suppressing cancer cell growth. According to the cytotoxicity criteria established by the National Cancer Institute (NCI), a compound is classified as a potent anticancer agent if it exhibits an IC_{50} value below 20 $\mu\text{g/mL}$.

The cytotoxic activity of *Myristica fragrans* Houtt leaf extract is presumed to be associated with its secondary metabolite content, including lignans, neolignans, and diarylnonanoids, which are known to exhibit various biological activities, including anticancer and chemo-preventive effects against cancer cell growth (Le *et al.*, 2024). Furthermore, previous studies have demonstrated that *Myristica fragrans* Houtt seed extract is capable of inducing apoptosis in B16-F10 malignant melanoma cells. These findings suggest that *Myristica fragrans* Houtt possesses a broad range of pharmacological properties (Lesmana *et al.*, 2021). In a previous study, the nutmeg leaf extract exhibited an inhibitory effect on MCF-7 cells of 26.85%, which was higher than that of the nutmeg seed extract, which achieved only 22.73% inhibition (Saputri *et al.*, 2024). These results indicate that nutmeg leaves possess greater cytotoxic activity than the seeds, potentially due to a higher content and/or greater efficacy of their bioactive compounds.

The findings of this study further support previous literature regarding the biological potential of *Myristica fragrans* Houtt as an effective anticancer and antioxidant agent, particularly against the MCF-7 breast cancer cell line. Evidence reported in 2024 confirmed that the

cytotoxic effects of both nutmeg seed and leaf extracts on these breast cancer cells are attributed to the presence of secondary metabolites, including neolignans, phenylpropanoids, and lignans. These compounds exert their effects through mechanisms such as apoptosis induction and suppression of cellular proliferation, further highlighting the potential of *Myristica fragrans* Houtt as a promising source of natural anticancer agents (Saputri *et al.*, 2024). Although the IC_{50} value of the nutmeg leaf extract obtained in the present study was relatively high, the results nevertheless indicate its biological potential as a chemo-preventive agent. The use of MCF-7 cells as an experimental model is also highly relevant, as this cell line represents the luminal A subtype of breast cancer, which is the most commonly diagnosed subtype worldwide (Horwitz *et al.*, 1975).

Therefore, the IC_{50} value of 415.80 $\mu\text{g/mL}$ indicates that the ethanolic extract of *Myristica fragrans* Houtt leaves exhibits an inhibitory effect on MCF-7 cells with a moderate level of cytotoxic activity. These findings suggest that the extract has potential for development as a chemo-preventive agent to prevent or suppress the growth of cancer cells. To further substantiate these findings, future studies should focus on the isolation and characterization of specific bioactive compounds, as well as the evaluation of their efficacy through *in vivo* studies. Such investigations are essential for elucidating the underlying biochemical mechanisms of action and for confirming the therapeutic potential of this promising anticancer candidate.

CONCLUSION

Based on the results of the analysis, it can be concluded that the ethanolic extract of *Myristica fragrans* Houtt leaves exhibits cytotoxic activity against MCF-7 breast cancer cells, with a moderate level of cytotoxicity ($IC_{50} = 415.80 \mu\text{g/mL}$). These findings indicate that nutmeg leaves have potential for development as a natural anticancer agent, although their efficacy remains moderate when compared with cisplatin, which was used as the positive control. We have also emphasized that further studies are needed to investigate the underlying mechanisms of action, evaluate its selectivity toward cancer cells, and confirm its potential anticancer activity.

CONFLICT OF INTEREST

All authors declare that there are no conflicts of interest.

AUTHORS' DECLARATION

All authors declare that the material presented in this article represents the original findings of the research conducted. The authors bear full responsibility for the accuracy and integrity of the data, analyses, and claims reported in this manuscript.

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