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Anticancer effectiveness of *Artemisia annua* ethanol extract against MDAMB-231 cancer cells

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ABSTRACT

Background: Breast cancer remains one of the leading causes of cancer deaths worldwide, with triple-negative breast cancer (TNBC) posing a significant therapeutic challenge due to its lack of hormonal receptors and resistance to conventional treatment. *Artemisia annua* L., a medicinal plant traditionally used in Chinese medicine, has received attention for its diverse bioactive compounds, including flavonoids, phenolic acids, and sesquiterpene lactones, which exhibit potential anticancer properties. This study aims to evaluate the in vitro cytotoxic effect of *A. annua* ethanol extract against MDA-MB-231 human breast cancer cells using the MTT assay. **Methods:** Extracts were prepared in 10% DMSO and tested at concentrations ranging from 0 to 1000 µg/mL. MDA-MB-231 cells were seeded in 96-well plates and incubated for 24 h before treatment. Post-treatment, cell viability was assessed via MTT assay, and absorbance was measured at 595 nm. The percentage of cytotoxicity was calculated, and the IC_{50} value was determined through linear regression analysis. **Results:** The results showed a clear cytotoxic response depending on the dose, with the extract achieving an IC_{50} value of 56.83 $\mu g \cdot mL^{-1}$ indicating a fairly strong correlation between concentration and cytotoxicity. These findings indicate that *A. annua* contains bioactive compounds that are able to inhibit cancer cell proliferation. **Conclusion:** In conclusion, Artemisia annua ethanol extract showed moderate cytotoxic activity against MDA-MB-231 cells, supporting its potential as a complementary therapeutic agent for TNBC. Further studies are needed to elucidate its molecular mechanisms and evaluate its efficacy in vivo.

Keywords: Breast cancer, MDAMB-231, Artemisia annua, IC50

INTRODUCTION

Cancer remains one of the most pressing global health challenges and a leading cause of mortality worldwide[1]. The transformation of normal cells into malignant ones involves multiple stages, including genetic alterations that activate oncogenes and suppress tumor suppressor genes, ultimately disrupting cell proliferation control. Each step in this carcinogenic process offers a potential target for anticancer agents, making the search for effective therapies increasingly vital[2].

Natural products have gained significant attention for their chemo-preventive properties, offering advantages such as lower toxicity, cost-effectiveness, and broad availability compared to conventional chemotherapy[4]. Artemisia annua, a medicinal herb traditionally used in Chinese medicine, has demonstrated promising anticancer activity against various cancer cell lines. Its extract

contains diverse bioactive compounds, including sesquiterpene lactones and phenolic constituents, which may contribute to its cytotoxic effects through mechanisms such as oxidative stress induction and metabolic disruption[3,5].

Recent studies have shown that *A. annua* exhibits selective cytotoxicity against cancer cells while sparing normal cells, suggesting its potential for therapeutic application. For instance, a study published in the Korean Journal of Physiology and Pharmacology reported that artemisinin derivatives induced apoptosis in human breast cancer cells via caspase activation and modulation of Bcl-2 family proteins. Similarly, network pharmacology analyses have identified multiple molecular targets of *A. annua* constituents, including MAPK, PI3K/AKT, and estrogen signaling pathways, which are frequently dysregulated in breast cancer.

Despite the growing body of in silico evidence, computational approaches such as molecular docking and network pharmacology cannot fully replicate the complexity of biological systems. Therefore, experimental validation remains essential to confirm the anticancer potential of herbal extracts. In vitro cytotoxicity assays, particularly the MTT assay, provide a reliable method to assess cell viability and quantify the antiproliferative effects of candidate compounds

This study aims to evaluate the anticancer effectiveness of ethanol extract of Artemisia annua against MDA-MB-231 human breast cancer cells using the MTT assay. By determining the concentration-dependent cytotoxicity and calculating the IC_{50} value, this research seeks to validate the therapeutic potential of *A. annua* and contribute to the development of plant-based interventions for triple-negative breast cancer, a subtype that lacks targeted hormonal therapies and remains clinically challenging[9].

MATERIALS AND METHODS

Material

Materials used: MDA-MB-231 cancer cells, DMEM + FBS culture medium, Artemisia annua ethanol extract, MTT reagent (3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide), DMSO, ELISA reader (595 nm), *Artemisia annua*.

Preparation of A. annua extract

Aerial parts of *Artemisia annua* (1 kg) were collected from a cultivation area in Tawangmangu. The plant material was identified by Mr. Santoso, S.Farm., Head of the Supporting, Research, and Product Provider Installation, Sarjito Hospital, Yogyakarta. The plant was carefully dried using a cabinet dryer to obtain 500 g. The extract was extracted with 96% ethanol (10 g/50 ml) twice at room temperature (27.50°C) with maceration for twenty-four hours. The extract was filtered and concentrated to dryness using a rotary evaporator at 45°C under vacuum, then stored at 4°C before use[10].

Cytotoxicity of A. annua extract on Breast Cancer (MDA-MB-231)

The tested extract dilutions were prepared using Dulbecco's Modified Eagle's Medium (DMEM). A stock solution of *Artemisia annua* ethanol extract was prepared in 10% DMSO diluted with double-distilled H_2O. The cytotoxic effect of the extract on MDA-MB-231 cells (a human breast cancer cell line) was evaluated using the MTT assay with slight modifications [1,2]. MDA-MB-231 cells were seeded into 96-well plates (100 µL/well at a density of 3×10^5 cells/mL) and incubated for 24 h at 37°C in a humidified atmosphere containing 5% CO_2. After incubation, the cells were treated

with various concentrations of the extract in triplicate and incubated for another 24 h. The culture medium was then discarded, and the wells were washed three times with sterile phosphate-buffered saline (PBS)[6]. MTT solution (20 µL of 5 45.28 µg·mL^{-1} stock) was added to the culture medium at a ratio of 1:10, then added to each well (100 µL) and incubated for 4 hours at 37°C, then the medium was discarded. The resulting formazan crystals were dissolved in 100 µL of DMSO, and incubated for 30 minutes[11]. Absorbance was measured using a multi-well plate reader at 595 nm. The percentage of cytotoxicity compared to untreated cells was calculated using the following equation: % Cytotoxicity = ((A_0 − A) / A_0) × 100, where A_0 is the absorbance of untreated cells and A is the absorbance of treated cells. The concentration causing 50% cytotoxicity (IC_{50}) was determined by plotting % cytotoxicity against the concentration of the extract.

RESULTS

The cytotoxic activity of *Artemisia annua* ethanol extract was quantitatively assessed using an MTT assay on MDA-MB-231 breast cancer cells over a 24-hour exposure period. The extract was tested at concentrations ranging from 0 to 1000 µg/mL. Absorbance readings were taken three times for each concentration, and the average value was corrected by subtracting the absorbance from the blank (0.0862). The resulting data were used to calculate the percentage of cell viability.

Table 1. %Viability of *A.annua* extract on (breast (MDA-MB-231) cell lines

A.annua extract (ppm)	Abs (595 nm)	%Viabilitas
1000	0.0856	2.4
500	0.0890	4.0
250	0.0925	5.7
125	0.1018	10.3
62,5	0.1473	32.6
31,25	0.2054	61.2
16,625	0.2440	80.1
0	0.2845	100

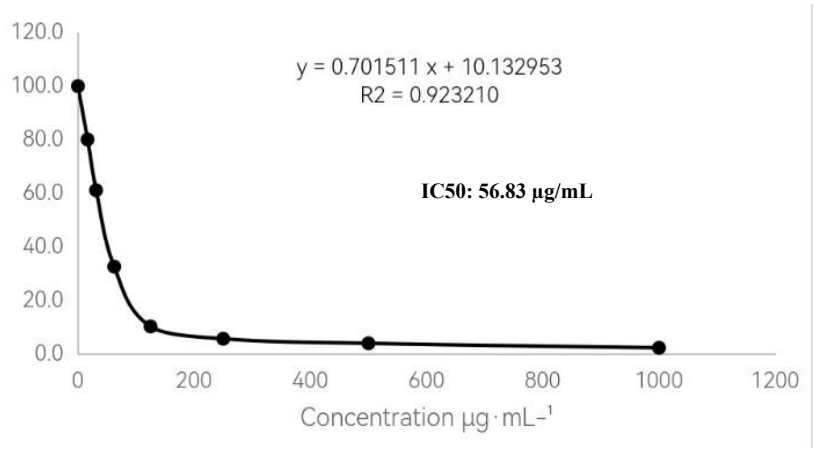

Figure 1. Dose response curve of the cytotoxic activity of *A.annua* extract on MDA-MB-231 cell lines

As shown in the data table, cell viability decreased progressively with increasing extract concentration. At a concentration of 31,25 µg/mL, viability remained above 80%, while at a concentration of 62,5 µg/mL, viability dropped below 50%, indicating a threshold for a significant cytotoxic response. The highest concentration tested (1000 µg/mL) resulted in less than 10% viability, confirming a potent cytotoxic effect. The dose-response relationship is further visualized in the corresponding graph, where cytotoxicity increases linearly with increasing concentration.

Artemisia annua ethanol extract was evaluated for its in vitro anticancer activity against human breast cancer cells (MDA-MB-231) using the MTT assay. The cytotoxicity profile demonstrated a clear dose-dependent effect, where increasing concentrations of the extract led to a progressive decline in cell viability. As shown in Table 1, the percentage of cytotoxicity increased linearly with concentration. The regression equation $y = 0.701511x + 10.132953$ with an R^2 value of 0.923, indicating a strong correlation. The calculated IC_{50} value was 56.83 $\mu g \cdot mL^{-1}$, suggesting that the extract possesses moderate cytotoxic potency against MDA-MB-231 cells after 24 hours of exposure.

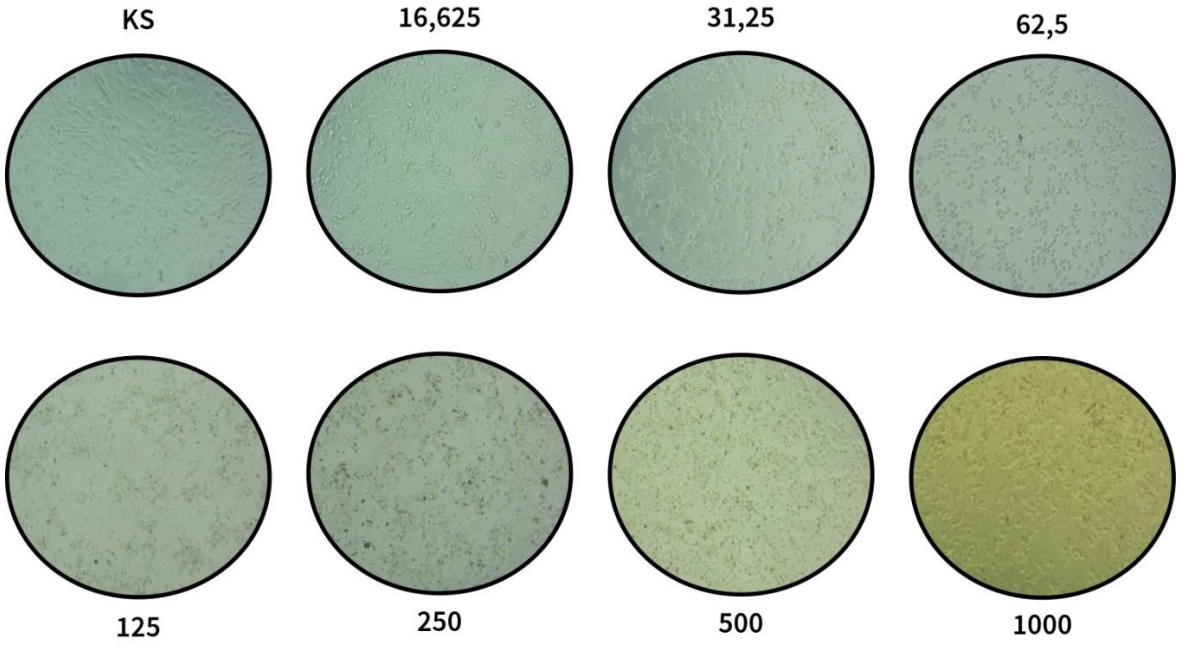

Figure 2. Effect of *A.annua* extract on MDA-MB-231 culture cells morphology

DISCUSSION

The cytotoxic activity observed in this study confirms that Artemisia annua ethanol extract exerts a concentration-dependent inhibitory effect on MDA-MB-231 breast cancer cells. The IC_{50} value of 56.83 $\mu g \cdot mL^{-1}$ indicates moderate potency, consistent with previous reports demonstrating selective cytotoxicity of *A. annua* against various cancer cell lines. This effect is likely attributed to the synergistic action of its phytochemical constituents, including artemisinin, flavonoids (e.g., chrysosplenol D, quercetin), and phenolic acids (e.g., caffeic and ferulic acid), which are known to interfere with cancer cell metabolism, induce oxidative stress, and promote apoptosis[15,16].

Among these compounds, artemisinin and its derivatives have been extensively studied for their ability to generate reactive oxygen species (ROS) in iron-rich environments such as cancer cells, leading to mitochondrial dysfunction and caspase-dependent apoptosis[16]. Chrysosplenol D, a

flavonol isolated from *A. annua*, has been shown to activate ERK1/2 signaling and induce autophagy in MDA-MB-231 cells, contributing to cell death through non-apoptotic pathways. Additionally, quercetin and luteolin modulate the PI3K/AKT and MAPK pathways, which are critical regulators of cell survival, proliferation, and migration in triple-negative breast cancer[17].

Beyond direct cytotoxicity, *A. annua* also influences the tumor microenvironment through modulation of cytokine signaling. Studies have shown that its polyphenolic fractions can downregulate pro-inflammatory cytokines such as IL-6 and TNF-α, which are often elevated in aggressive breast cancer phenotypes and contribute to tumor progression, angiogenesis, and immune evasion. By suppressing NF-κB activation and reducing cytokine-mediated signaling, *A. annua* may help restore immune surveillance and inhibit metastatic potential[20].

Furthermore, the extract's ability to interfere with adhesion molecules such as VCAM-1 and downregulate β-catenin and MMP-9 expression suggests a role in limiting cancer cell invasiveness and stem-like behavior[22,23]. These molecular targets are particularly relevant in MDA-MB-231 cells, which exhibit high migratory and invasive capacity[25]. The multi-targeted nature of A. annua—acting on both intracellular signaling and extracellular cytokine networks—positions it as a promising candidate for integrative cancer therapy, especially in subtypes like TNBC that lack effective targeted treatments.

These findings suggest that the bioactive constituents of *A. annua*, particularly its polyphenolic and sesquiterpene compounds, may contribute to the inhibition of cancer cell proliferation through mechanisms involving oxidative stress and disruption of cellular metabolism. Given its natural origin, reduced toxicity, and complex phytochemical profile, *Artemisia annua* holds promise as a complementary therapeutic candidate for breast cancer, especially for triple-negative subtypes that lack targeted hormonal treatment options. Further studies are warranted to elucidate the molecular pathways involved and to explore its potential in combination with existing chemotherapeutic agents.

CONCLUSION

The present study demonstrated that the ethanol extract of Artemisia annua exerts a concentration-dependent cytotoxic effect on MDA-MB-231 human breast cancer cells. Through in vitro evaluation using the MTT assay, the extract achieved an IC_{50} value of 56.83 $\mu g \cdot mL^{-1}$ after 24 hours of exposure, indicating moderate anticancer potency

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Competing Interests

The authors declare that there is no conflict of interest.

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