narra j

Original Article

Cytotoxic activity of ethyl acetate extract of *Chromolaena odorata* on MCF7 and T47D breast cancer cells

Hanifah Yusuf[1*], Hijra Novia[1] and Marhami Fahriani[2]

[1]Department of Pharmacology, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia; [2]Medical Research Unit, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia

*Corresponding author: hans_yusuf1104@usk.ac.id

Abstract

One of the most cancers in women that can be fatal is breast cancer. Radiation therapy, chemotherapy, or a combination of the two are often used to treat cancer, and these treatments tend to modify the immune system and weaken defences. Using natural compounds from plants has become a research interest to prevent cancer cell development. The aim of this study was to determine the anticancer activities of ethyl acetate extract of *Chromolaena odorata* (EACO) against breast cancer cells (MCF-7 and T47D). The viability of the cells was determined by the MTT colorimetry assays. The apoptosis test was performed by using flow cytometry. The IC_{50} value for MCF-7 cells was 218.78 µg/mL and 307.61 µg/mL for T47D. The extract acted selectively against breast cancer cells, with selectivity indexes against MCF-7 and T47D were 12.77 and 9.08, respectively. The viable cells of T47D cells were decreased from 85±36.5% (24 hours) to 54±34% (48 hours) after treatment with IC_{50} of EACO. Significant decrease of the MCF-7's viable cells were observed between 48 and 72 hours after treatment with IC_{50} (68.5±17.7% to 51.01±12.1%, respectively). Apoptosis assay showed that T47D and MCF-7 cells were mainly in the necrosis stage (83.35±0.49% and 95.15±1.76%, respectively). This study suggested that ethyl acetate extract of *C. odorata* is promising to be developed as an anticancer agent.

Keywords: Breast cancer, *Chromolaena odorata*, apoptosis, anti-cancer, cytotoxic activity

Introduction

Breast cancer was the most frequently diagnosed cancer in women in 2020, with a prevalence of 11.7% among all cancer cases [1]. An estimated 685,000 women died from breast cancer in 2020, making breast cancer the fifth-leading cause of cancer-related deaths worldwide [1]. Incidence rate for breast cancer was 88% higher in developed countries than in developing countries (55.9 vs 29.7 per 100,000) [1]. The mortality rate from female breast cancer was 17% higher in developing countries than in developed countries (15.0 vs 12.8 per 100,000) [1]. Despite a 0.5% annual increase in incidence, the mortality rate of breast cancer cases has dropped by 43% in the United States since 1989 [2]. However, in 2020, the incidence of breast cancer in Indonesia reached 68,858 cases (16.6%) out of a total of 396,914 new cases of cancer in Indonesia, with 22,430 deaths recorded [1].

Breast cancer is usually treated with combination of surgery, radiation, and medication (hormonal therapy, chemotherapy, and targeted biological therapy) [3]. These treatments may

https://narraj.org/

Received: September 16, 2023 - Accepted: November 7, 2023 - Published online: November 20, 2023

prevent growth and inhibit the spread of cancer. However, cancer treatment with radiation, chemotherapy, or a combination of the two tends to alter the body's defense system, lowering immunity and resulting in death [4]. In contrast, natural products isolated from medicinal plants have been used to treat various diseases since ancient times. Since most target-specific anticancer drugs failed to achieve the desired outcome, new therapies using natural products have become significant. As a potential therapeutic agent for cancer, natural products are readily available, inexpensive, with minimum cytotoxicity against normal cells [5]. The use of natural materials, predominantly plants, has become a trend by world pharmacologists to prevent the development of cancer cells and repair DNA and RNA damaged during gene mutations through signalling in the cell cycle pathway [6].

The perennial shrub *Chromolaena odorat*a (L.) R.M. King & H. Robinson, also known as *Eupatorium odoratum* L., is a member of the Asteraceae family and has been previously used in traditional Chinese medicine [7]. This plant grows to a height of 2–3 m [8], native to the Caribbean islands and southern Florida and Texas [9]. *C. odorata* is a plant that thrives on various soil types and in various vegetation types, such as grasslands, arid bushveld, and forests, with an annual rainfall of less than 500 mm. The first introduction to Southeast Asia was most likely through the Calcutta Botanic Garden and it most likely spread throughout Indonesia via live cattle shipments [10].

C. odorata has various beneficial effects such as the leaves were used on wounds in Thailand to stop bleeding [7]. Several studies have reported *C. odorata* having antimicrobial [11,12], anti-biofilm [13], anti-hepatotoxic [14,15], and antiproliferative properties [16-18]. Additionally, this plant contains chemical compounds that aid in limiting the development of pathogenic microorganisms and scavenged free radicals and possess selective anticancer properties [18].

C. odorata, as an anticancer agent, has been studied against various cancer cell lines, such as cervical, breast, colorectal, and liver cancer [19-24]. Studies have revealed the cytotoxic mechanism of this plant against the Ca151 breast cancer cell type by inhibiting *Bcl2* expression and inducing apoptosis by inhibiting apoptosis signal kinase 1 against colorectal cancer [17,25]. However, previous studies only tested ethanol extract of this plant [23]. The aim of this study was to explore the potential cytotoxic activity of ethyl acetate extract of *C. odorata* (EACO) on breast cancer cells.

Methods

Plant material

C. odorata leaves were collected in Aceh Besar District, Aceh Province, Indonesia. The plants were identified by the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh, Indonesia (No B/435/UN11.1.8.4/TA.00.01/2020).

Extraction of *C. odorata* leaves

The extraction method of *C. odorata* leaves was previously described [21]. Briefly, the leaves were collected, washed, and dried. Then, they were ground with an electric grinder. The dried powdered leaves weighed five kilograms and were macerated in a 1:5 ratio with two different solvents of varying polarity. Leaves were macerated in n-hexane, stirred every four hours, and filtered every 24 hours. The n-hexane filtrate was collected after 72 hours, and vacuum evaporated at 40°C in a vacuum-rotating evaporator to acquire the viscous n-hexane extract. The n-hexane waste was dried and macerated for 72 hours in ethyl acetate, stirred every four hours, and filtered every 24 hours. The ethyl acetate extract of *C. odorata* leaves (EACO) was then collected after 72 hours.

Cell culture

The breast cancer cells, T47D (ductal carcinoma) and MCF-7 (adenocarcinoma), were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum, 2% penicillin-streptomycin, and fungizone 0.5% (Gibco, USA). The cells were kept in a humidified atmosphere (5% CO_2) at 37°C [24].

Viability assay

The viable cells of T47D and MCF-7 were evaluated by MTT colourimetric assay. This experiment was conducted in triplicate. The cells were cultured in 96-well microplates containing 1x10^4 cells/100 µL. Then, the cells were incubated at 37^{o}C with 5% CO_2 for 24 hours. Vero cells acted as the negative control, MCF-7, and T47D cells were treated with EACO as the treatment, MCF-7 and T47D cells were treated with doxorubicin (DOXO) as the positive control, and well-seeded growth medium-only served as the medium (blank) sample. Four 96 wells-microplates were cultured with 1x10^4 cells/100 µL of cells or medium and incubated at 37°C in a 5% CO2 and 90% humidity environment for 24 hours. The culture media was discarded after confluent cells (70%–80%) were reached. Various concentrations of EACO (treatment group) were added to each well: 500, 250, 125, 62.5, 31, 15.63, and 7.8 µg/mL. Afterwards, 10µL MTT solution was added into each well, followed by four hours of incubation in a CO_2 incubator at 37^{o}C until purple formazan crystals were formed. The reaction was then stopped by adding a 10% SDS stopper solution in 0.1 N hydrochloride (HCl) acid, and the plates were incubated in the dark overnight. The absorbance of the plates, which was measured using a microplate enzyme-linked immunosorbent assay (ELISA) reader at 595 nm, was then used to quantify the proportion of live cells.

The percentage of viable cells = (tested cell absorbance - medium absorbance) / (control cell absorbance - medium absorbance) x 100%. The half-maximal inhibitory concentration (IC_{50}) value was calculated using the percentage of the viable cells. The IC_{50} value represents the EACO concentration that can cause 50% of cell deaths. The selectivity index (SI) was then calculated using the IC_{50} values: SI ratio = IC_{50} of Vero cells/ IC_{50} of cancer cells. The SI ratio >3 means the EACO treatment has a selective effect only on the breast cancer cells (MCF-7 and T47D cells).

Cell proliferation assay

The antiproliferative activity of EACO was also examined by the MTT colorimetry assay. A 96-well plate was seeded with 100 µL of T47D and MCF-7 cells (70–80% confluent) at a 1.5x10^4 cells/well density and a growth medium. After that, the cells underwent a 24-hour incubation. Then, 100 µL of the ½ IC_{50}, IC_{50}, and 2 x IC_{50} of EACO were applied to the cells for 72 hours. The plate was observed every 24 hours. Then, 100 µL of MTT 0.5 mg/mL was used to treat the plate, and the plate was allowed to sit for four hours until a purple formazan crystal developed. The plate was filled with the stopper solution (10% SDS in 0.1N HCl acid) and then incubated overnight in the dark. Then, the proliferation was determined by reading the plates' absorbance and converting the result into the percentage of viable cells. All proliferation test was conducted in triplicate.

Apoptosis assay

T47D and MCF7 cells were seeded into 6-well plates with a cell density of 5 x 10^4 cells/well. After incubated for 24 hours in a 5% CO_2 incubator, cells were treated with *C.odorata* leaf extract with a concentration of 2 x IC_{50}, then incubated again for 24 hours. Then, the cells were harvested using 0.25% trypsin-EDTA, centrifuged, and washed with PBS. Afterwards, cells were resuspended in 500 µL of Annexin V buffer and then treated with Annexin V and propidium iodide (PI) for 10 minutes at room temperature and in a dark place. To assess the homogenized cell suspension, BD FACS Calibur Flow cytometer (Becton Dickinson, California, US) was used, and the result was analyzed by Modfit Lt. 3.0 (Verity Software House, Maine, US). The apoptosis assay was conducted in triplicate. The principle of apoptosis assay using Annexin V and PI were described as follows. The membrane phospholipid phosphatidylserine (PS) of apoptotic cells is translocated from the inner to outer leaflet of the plasma membrane, exposing PS to the external cellular environment. Annexin V, which conjugated to flourochromes, binds to exposed apoptotic cell surface PS and acts as a sensitive probe for flow cytometric assay of apoptotic cells.

After PS translocation, the membrane lose its integrity in the later phases of cell death caused by either apoptotic or necrotic processes. Therefore, in addition to Annexin V, propidium iodide (PI) was utilised to identify early and late apoptotic cells. Viable cells with intact membranes exclude PI, whilst the membranes of apoptotic cells are permeable to PI. Healthy cells are both Annexin V and PI negative, while cells that are in early apoptosis are Annexin V positive and PI

negative, and cells in late apoptosis are Annexin V and PI positive. Cells that already in necrosis are Annexin V negative and PI positive.

Statistical analysis

Results were presented as mean±SD. Nonlinear regression analysis was used to determine the IC_{50} of EACO for MCF-7 and T4D7 cells. Analysis of variance (ANOVA), followed by a Tukey posttest, was used to analyze the viability and the proliferation of cancer cells against different concentrations of EACO. Multiple t-tests were used to analyze cancer cells' apoptosis compared to control when treated with different levels of IC_{50} of EACO. A $p<0.05$ was regarded as statistically significant. Statistical analysis was performed using Graph Pad Prism v.8.0.2 (GraphPad Software, CA, USA).

Results

IC_{50} values of EACO on T47D and MCF-7 cell lines

Our data indicated a dose-dependent manner of anticancer activity of EACO against MCF-7, T47D, and Vero cells (**Figure 1**). The concentration of 500 µg/mL of EACO reduced the viability of the MCF-7 and T47D cells to 1.2% and 5.4%, respectively ($p<0.05$), whereas the viable cell of Vero was 61.3%. Meanwhile, the viability of the cells was above 50% at other EACO concentrations.

The MCF-7, T47D, and Vero cells' viability were also decreased after treatment with DOXO. Treatment with 100 µg/mL DOXO resulted in 16.5% of MCF-7, 23.8% of T47D, and 5.7% of Vero viable cells, respectively. The IC_{50} of EACO on MCF-7 was 218.78 µg/mL, and T47D cells were 307.61µg/mL. The IC_{50} of Vero cells after treatment with EACO was 2792.5 µg/mL. The EACO SI against MCF-7 and T47D were 12.77 and 9.08, respectively. The IC_{50} of DOXO against MCF-7 and T47D were 5.4 µg/mL and 1.75 µg/mL, respectively.

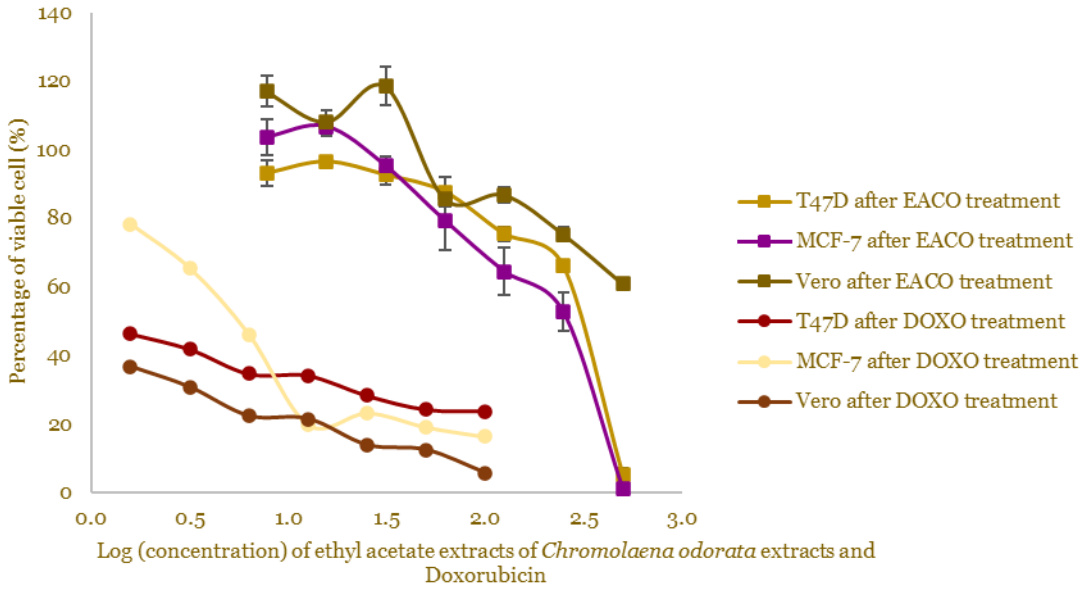

Figure 1. The viability of MCF-7, T47D, and Vero cells after being treated with ethyl acetate extract of *Chromolaena odorata* (EACO) and doxorubicin (DOXO).

Proliferation of T47D and MCF-7 cells against EACO

The viable cells of T47D cells after treatment with ½ IC_{50}, IC_{50}, and 2 x IC_{50} of EACO at 24 hours were 18.1±9.37%, 85±6.5%, and 100±4.9%, respectively (**Figure 2A**). The T47D cells were decreased after 48 hours (1/2 IC_{50}: 10.7±6.5%, IC_{50}: 54±3.9%, 2 x IC_{50}: 81±7.7%), although not significantly differ from those at 72 hours (1/2 IC_{50}: 12.7±3.4%, IC_{50}: 52.3±2.2%, 2xIC_{50}: 117.1±3.3%) (**Figure 2A**).

The viable cells of MCF-7 were at 20.0±1.8%, 62.7±4.1%, and 99.4±9.4% after 24 hours treatment with ½ IC_{50}, IC_{50}, and 2 x IC_{50}, respectively (**Figure 2B**). After 48 hours, the MCF-7 cells decreased to 10.0±6.1% when treated with ½ IC_{50}. However, treatment with IC_{50} and 2 x IC_{50} resulted in 68.5±7.7%, and 102±5.2%, respectively with $p<0.05$ in both comparisons. The viable cells after 72 hours of treatment with ½ IC_{50} (19.9±9.6%) were also significantly lower than 2xIC_{50} (86.7±4.5%) with $p<0.05$ (**Figure 2B**).

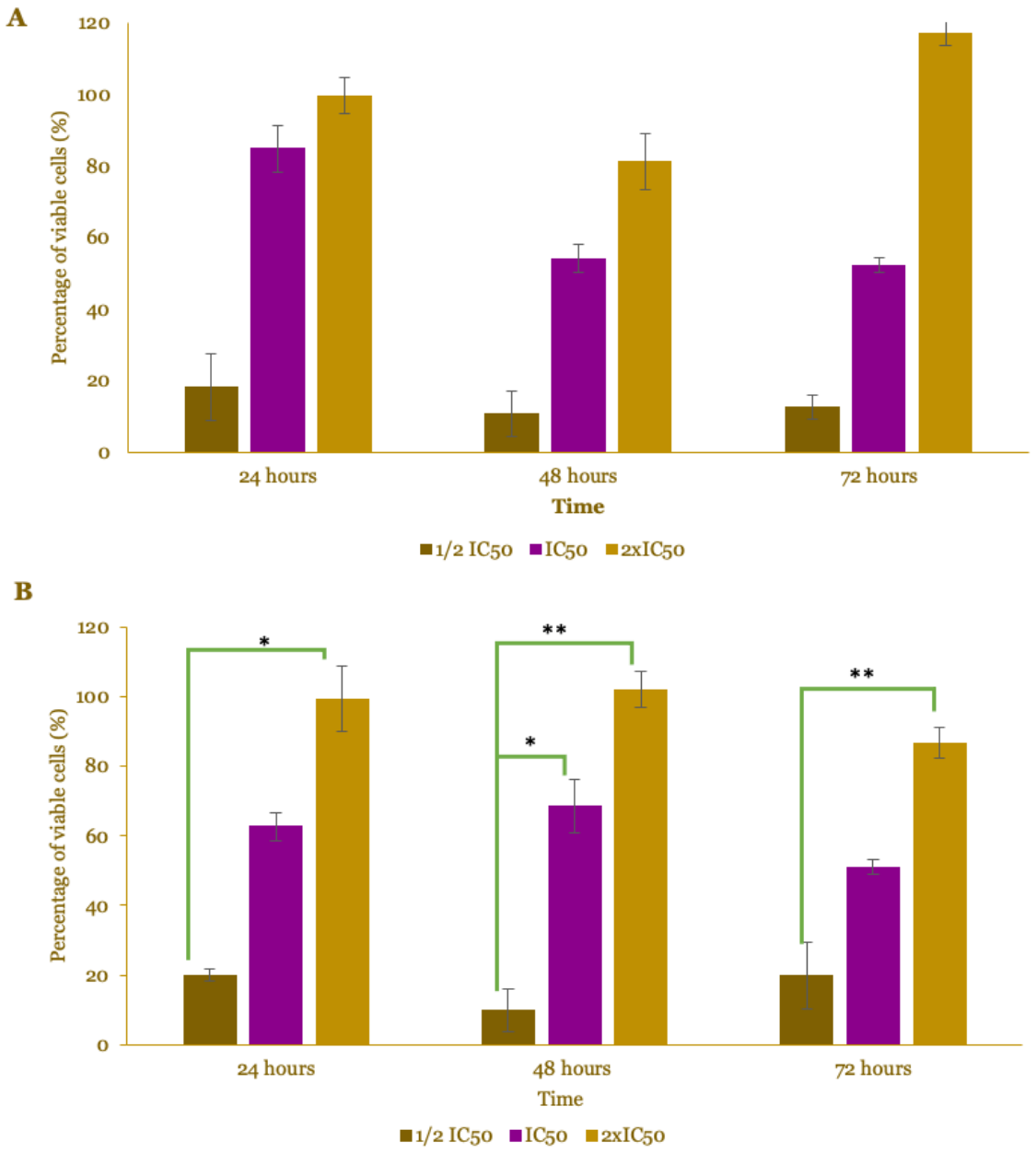

Figure 2. The proliferation of T47D (A) and MCF-7 (B) cells after 24-, 48- and 72-hours incubation following treatment with ½ IC_{50} (dark brown), IC_{50} (green), and 2xIC_{50} (light brown) of ethyl acetate extract of *Chromolaena odorata* (EACO).

The apoptosis induction test of T47D and MCF-7 cells after treatment with EACO

In the apoptosis assay for T47D cells, the viable cells of control (92.45±0.21%) were higher than T47D cells treated with 2 x IC50 of EACO (4.85±0.21%) with $p<0.001$ (**Figure 3A**). The number of T47D cells undergoing the early apoptosis stage after treatment with 2 x IC_{50} of EACO (6.20±0.14%) was higher than the control (2.7±0.14%), $p<0.01$. A higher number of T47D cells in the late apoptosis stage was observed after 2 x IC_{50} of EACO (7.1±0.42%) compared to the

control (p<0.05). Necrosis T47D cells after treatment with EACO were higher (83.35±0.49%) than the control (3.1±0.14%), with p<0.001 (**Figure 3A**).

The viable cells of MCF-7 (0.5±0.42%) after treatment with 2 x IC_{50} of EACO were lower than the control (82.75±0.35%) with p<0.001 (**Figure 3B**). The difference between MCF-7 and control cells during the early apoptosis stage was insignificant. However, the number of control cells in the late apoptosis stage (6.6±0.14%) was higher than MCF-7 (3.9±0.14%), p<0.05. The dead cells of MCF-7 (95.15±1.76%) after 2 x IC_{50} of EACO treatment were higher than the control (8.25±0.07%) with p<0.001 (**Figure 3B**).

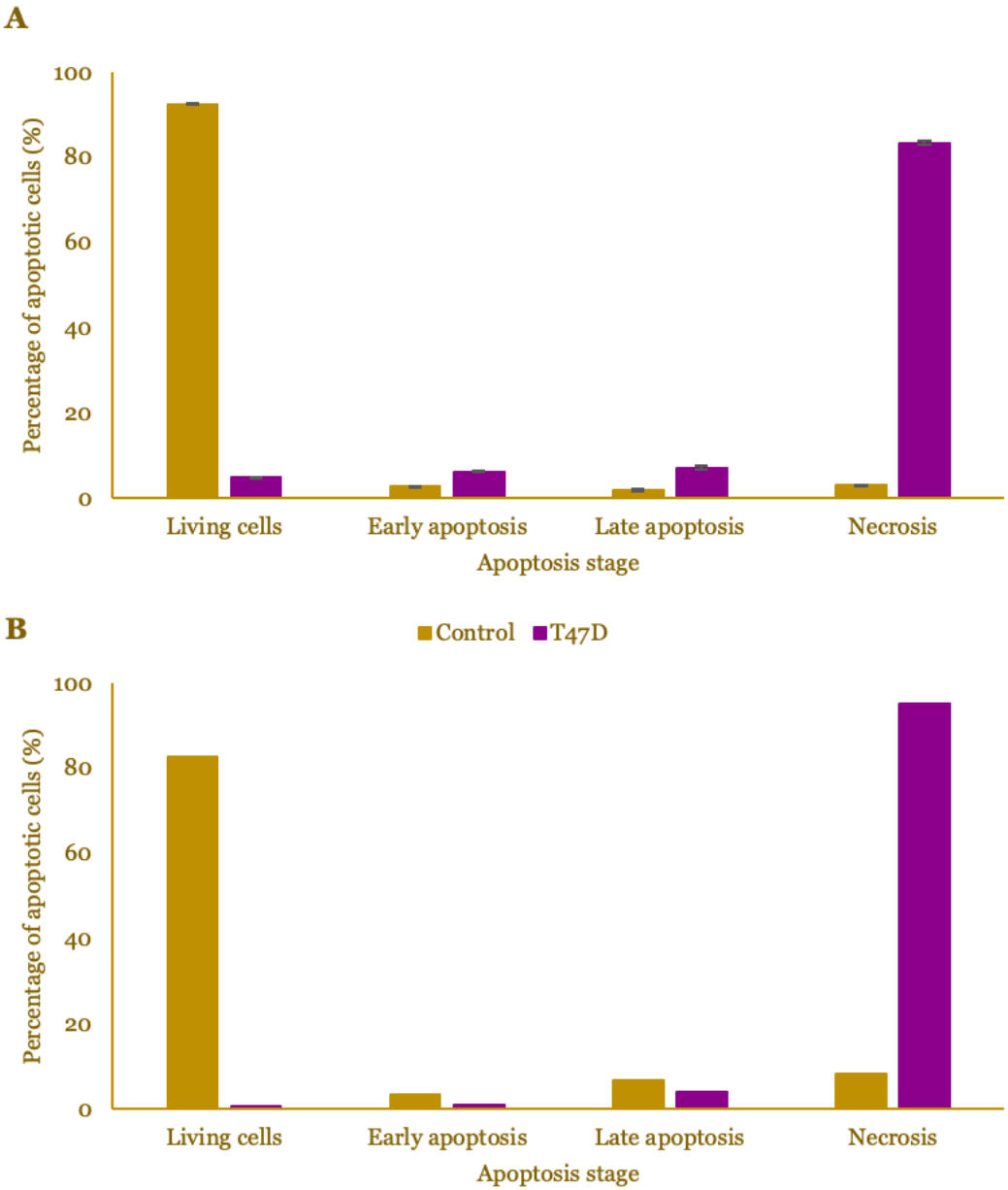

Figure 3. The apoptosis analysis using flow cytometry on (A) T47D and (B) MCF-7 cells using 2xIC_{50} of acetate extract of *Chromolaena odorata* (EACO).

Discussion

Based on a previous study, the anticancer activity of *C. odorata* was detected from its ethanol extract in a dose-dependent manner [23]. Similarly, this study showed that treatment with 500 µg/mL EACO selectively decreased the viable cells of MCF-7 and T47D by more than 94% while preserving the Vero cells (61.3%). This result was better than treatment with 100 µg/mL DOXO, which decreased not only the viable cells of MCF-7 and T47D but also Vero cells (5.7%). The SI

ratio of EACO for MCF-7 and T47D cells were also more than three, indicating that EACO was safer for normal cells than DOXO.

More than 190 different phytochemicals have been isolated from *C. odorata* and identified, such as fatty acids, triterpenoids, diterpenes, sesquiterpenes, essential oils, alkaloids, anthracene derivatives, flavonoids, stilbenes, and derivatives of diterpene [26]. Most flavonoids and phenolic acids, such as odoratenin, persicogenin, and (Z)-p-coumaric acid, were found in the aerial parts and leaves of *C. odorata* [27,28]. Meanwhile, steroids and triterpenoids (poriferasterol, stigmasterol, acetyl oleanolic acid, and ursolic acid) were commonly isolated from the stem, flowers, and roots and sometimes found in the leaves and aerial parts [28,29]. Using dichloromethane extract, the alkaloids and anthraquinones, such as 7-angeloylretronecine, 3-hydroxy-1,2,4-trimethoxy-6-methylanthraquinone, austrocortinin, and rinderine, were commonly found in the entire plant and flowers [30]. Straight-chain alkanes and fatty acids were commonly identified from this plant's aerial parts, leaves, stem, and flower, which include (S)-coriolic acid, linoleamide, and hexacosanol [31,32]. Other compounds such as terpenoids (radicol and cadalene), sesquiterpenoids (germacrene D) and phenolic glycosides (citrusin) were found in the aerial parts and the whole plants of *C. odorata* [25,28,29,33]. Alpha amyrin, which was previously detected in this plant, is known to inhibit tumor necrosis factor-α (TNF-α), which prevents cancer development [23,34]. The α, β-amyrin is a triterpenoid that functions as an antipyretic, anti-inflammatory, hepatoprotective, and analgesic. This compound is efficacious in preventing the development of pancreatic infection and acts as an anti-inflammatory and antioxidant agent [35].

Our previous studies showed that *C. odorata* had anticancer activity against several cancer cell lines [20-24]. Flavonoid compounds found in *C. odorata* can potentially inhibit the proliferation of cancer cells. A study stated that flavonoids inhibit cyclin-dependent kinases (CDKs), which are essential for cell growth and division, block angiogenesis, and modulate MDR1 activity [36]. Flavonoids are essential in angiogenesis and the reactive oxygen species (ROS) activity of tumor cells, preventing cancer cell proliferation [37]. Several phenolic compounds, alkaloids, terpenoids, and glycosides of *C. odorata* have been identified based on the phytochemical analysis [23]. The functional properties of these compounds modulate the scavenging enzyme activities of ROS, playing a role in stopping the cell cycle by inducing autophagy and apoptosis to suppress cancer cell proliferation and invasion [38].

In addition, a study showed that *C. odorata* has anticancer characteristics that increase apoptosis in Cal51 breast cancer cells through its synergistic interaction with the Bcl2 inhibitor ABT737 [25]. *C. odorata* induces apoptosis by triggering the p53 pathway, alerting a cell cycle checkpoint to eliminate cells considered harmful to the immune system. The checkpoint cycle controls excessive cell growth and property changes [39]. Pro-non-apoptotic protein receptors hinder the cell cycle checkpoint system by increasing the response to checkpoint sensor proteins during the pathogenesis of cancer cells [40]. Flavonoids typically increase caspases, which catalyze proteolysis and inactivate structural proteins that control cancer cell growth like gelsolin, poly-ADP-ribose polymerase (PARP), cytokeratins, and DNA fragmentation factors 45 kDa (DFF45), resulting in morphological and biochemical changes [41].

This study did not isolate the pure compund of anticancer agent from ethyl acetate extracts of *C. odorata*. Further study on isolating the anticancer agent and study the molecular binding with breast cancer receptor is recommended.

Conclusion

The ethyl acetate extract of *C. odorata* showed promising results as an anticancer against breast cancer. The extract acts selectively against breast cancer cells while preserving the normal cells based on an in-vitro assay. Furthermore, the compound's anticancer activity works by preventing the proliferation and inducing apoptosis of breast cancer cells.

Ethics approval

This study has received ethical approval from the Ethics Commission, Faculty of Medicine, Universitas Lambung Mangkurat, Banjarmasin, Indonesia, 239/KEPK/FK UNLAM/VI/2020.

Acknowledgments

We would like to thank the Department of Parasitology, Universitas Gadjah Mada for the technical support.

Competing interests

All the authors declare that there are no conflicts of interest.

Funding

This study received no external funding.

Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

How to cite

Yusuf H, Novia H, Fahriani M. Cytotoxic activity of ethyl acetate extract of *Chromolaena odorata* on MCF7 and T47D breast cancer cells. Narra J 2023; 3 (3): e326 - http://doi.org/10.52225/narra.v3i3.326.

References

1. Sung H, Ferlay J, Siegel RL, *et al*. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: Cancer J Clin 2021; 71(3):209-249.
2. Giaquinto AN, Sung H, Miller KD, *et al*. Breast Cancer Statistics, 2022. CA: Cancer J Clin 2022; 72(6):524-541.
3. Łukasiewicz S, Czeczelewski M, Forma A, *et al*. Breast cancer—Epidemiology, risk factors, classification, prognostic markers, and current treatment strategies—an updated review. Cancers 2021; 13(17):4287.
4. Rani V, Prabhu A. Combining angiogenesis inhibitors with radiation: advances and challenges in cancer treatment. Curr Pharm Des 2021; 27(7):919-931.
5. Dutta S, Mahalanobish S, Saha S, *et al*. Natural products: An upcoming therapeutic approach to cancer. Food and Chemical Toxicology 2019; 128:240-255.
6. Rahman MM, Sarker MT, Tumpa MAA, *et al*. Exploring the recent trends in perturbing the cellular signaling pathways in cancer by natural products. Frontiers in Pharmacology 2022; 13.
7. Yang J-N, Yi J-L, Zou J-H, *et al*. Flavonoidal alkaloids from the flowers of *Chromolaena odorata* (L.) R.M.King & H.Rob. Nat Prod Res 2023:1-9.
8. Zachariades C, Day M, Muniappan R, *et al*. *Chromolaena odorata* (L.) King and Robinson (Asteraceae). In: *Biological Control of Tropical Weeds Using Arthropods*. edn. Edited by Raman A, Reddy GVP, Muniappan R. Cambridge: Cambridge University Press; 2009: 130-162.
9. Kato-Noguchi H, Kato M. Evolution of the secondary metabolites in invasive plant species *Chromolaena odorata* for the defense and allelopathic functions. Plants 2023; 12(3):521.
10. Global Invasive Species Database. Species profile: C*hromolaena odorata*. 2023.
11. Mondal KC, Bhargava D, Shivapuri JN, *et al*. In vitro antigonorrhoeal activity and extraction of chemical constituents from the leaves of *Chromolaena odorata* (Lin.) locally known as BANMARA. Int J Chem Anal Sci 2012; 3(7):1487-1495.
12. Atindehou M, Lagnika L, Guérold B, *et al*. Isolation and identification of two antibacterial agents from *Chromolaena odorata* L. active against four diarrheal strains. Adv Microbiol 2013; 3(01):115-121.
13. Yahya M, Ibrahim M, Zawawi W, *et al*. Biofilm killing effects of *Chromolaena odorata* extracts against *Pseudomonas aeruginosa*. Res J Phytochem 2014; 8(3):64-73.
14. Alisi CS, Onyeze GOC, Ojiako OA, *et al*. Evaluation of the protective potential of *Chromolaena odorata* Linn. extract on carbon tetrachloride-induced oxidative liver damage. Int J Biochem Res Rev 2011; 1(3):69.
15. Asomugha R, Okafor P, Ijeh I, *et al*. Hepatic effects of aqueous extract of *Chromolaena odorata* in male Wistar albino rats. Pharmacologyonline 2014; 1:127-136.
16. Mutalib NA, Bakri ZM, Jalil KA, *et al*. Combination treatment of *Chromolaena odorata* ethanolic extract with cisplatin against breast cancer cell lines MDA-MB-231 and MCF-7. Cell; 100:1.

17. Omoboyowa DA, Iqbal MN, Balogun TA, *et al*. Inhibitory potential of phytochemicals from *Chromolaena odorata* L. against apoptosis signal-regulatory kinase 1: A computational model against colorectal cancer. Comput Toxicol 2022; 23:100235.
18. Hu J, Qi Q, Zhu Y, *et al*. Unveiling the anticancer, antimicrobial, antioxidative properties, and UPLC-ESI-QTOF-MS/GC–MS metabolite profile of the lipophilic extract of siam weed (*Chromolaena odorata*). Arab J Chem 2023; 16(7):104834.
19. Selvanathan J, Sundaresan S. Cytotoxicity effects on negative breast and colon cancer cell lines of *Chromolaena odorata*. J Compos Theory 2020; 13:223-243.
20. Yusuf H, Fahriani M. Anticancer activity and apoptotic induction of *Chromolaena odorata* Linn leaves extract and fractions on hepatocellular carcinoma cell lines (HepG2). J Natural 2022; 22(1):57-67.
21. Yusuf H, Fahriani M, Murzalina C. Anti proliferative and apoptotic effect of soluble ethyl acetate partition from ethanol extract of *Chromolaena odorata* linn leaves against hela cervical cancer cell line. Asian Pac J Cancer Prev 2022; 23(1):183-189.
22. Yusuf H, Fahriani M, Murzalina C, *et al*. Inhibitory effects on HepG2 cell proliferation and induction of cell cycle arrest by *Chromolaena odorata* leaf extract and fractions. Pharmacia 2022; 69(2):377-384.
23. Yusuf H, Husna F, Gani BA, *et al*. The chemical composition of the ethanolic extract from *Chromolaena odorata* leaves correlates with the cytotoxicity exhibited against colorectal and breast cancer cell lines. J Pharm Pharmacogn Res 2021; 9(3):344-356.
24. Yusuf H, Kamarlis RK, Yusni Y, *et al*. The anticancer activity of ethanol extract of *Chromolaena odorata* leaves in 7, 12-Dimethylbenz [a] anthracene in (DMBA) induced breast cancer Wistar rats (*Rattus novergicus*). Pharmacia 2021; 68(2):493.
25. Kouamé PBK, Jacques C, Bedi G, *et al*. Phytochemicals isolated from leaves of *Chromolaena odorata*: impact on viability and clonogenicity of cancer cell lines. Phytother Res 2013; 27(6):835-840.
26. Ojo O, Mphahlele MP, Oladeji OS, *et al*. From wandering weeds to pharmacy: An insight into traditional uses, phytochemicals and pharmacology of genus *Chromolaena* (*Asteraceae*). J Ethnopharmacol 2022; 291, :115-155.
27. Putri DA, Fatmawati S. A new flavanone as a potent antioxidant isolated from *Chromolaena odorata* L. leaves. Evid Based Complementary Altern Med 2019; 2019::1453612.
28. Pel P, Chae H-S, Nhoek P, *et al*. A stilbene dimer and flavonoids from the aerial parts of *Chromolaena odorata* with proprotein convertase subtilisin/kexin type 9 expression inhibitory activity. Bioorg Chem 2020; 99:103869.
29. Zhang M-L, Irwin D, Li X-N, *et al*. PPARγ agonist from *Chromolaena odorata*. J Nat Prod 2012; 75(12):2076-2081.
30. Biller A, Boppré M, Witte L, *et al*. Pyrrolizidine alkaloids in *Chromolaena odorata*. Chemical and chemoecological aspects. Phytochemistry 1994; 35(3):615-619.
31. Gade S, Rajamanikyam M, Vadlapudi V, *et al*. Acetylcholinesterase inhibitory activity of stigmasterol & hexacosanol is responsible for larvicidal and repellent properties of *Chromolaena odorata*. Biochim Biophys Acta - Gen Subj 2017; 1861(3):541-550.
32. Hanh TTH, Hang DTT, Van Minh C, *et al*. Anti-inflammatory effects of fatty acids isolated from *Chromolaena odorata*. Asian Pac J Trop Med 2011; 4(10):760-763.
33. Koba K, Nénonéné AY, Catherine G, *et al*. Chemical composition and cytotoxic activity of essential oil of *Chromolaena odorata* L. growing in Togo. J Essent Oil-Bear Plants 2011; 14(4):423-429.
34. de Almeida PD, Boleti AP, Rüdiger AL, *et al*. Anti-Inflammatory Activity of Triterpenes Isolated from Protium paniculatum Oil-Resins. Evid Based Complementary Altern Med 2015; 2015, :293768.
35. Melo CM, Carvalho KM, Neves JC, *et al*. alpha,beta-amyrin, a natural triterpenoid ameliorates L-arginine-induced acute pancreatitis in rats. World J Gastroenterol 2010; 16(34):4272-4280.
36. Batra P, Sharma AK. Anti-cancer potential of flavonoids: recent trends and future perspectives. 3 Biotech 2013; 3(6):439-459.
37. Reuter S, Gupta SC, Chaturvedi MM, *et al*. Oxidative stress, inflammation, and cancer: How are they linked? Free Radic Biol Med 2010; 49(11):1603-1616.
38. Kopustinskiene DM, Jakstas V, Savickas A, *et al*. Flavonoids as anticancer agents. Nutrients 2020; 12(2).
39. Barnum KJ, O'Connell MJ. Cell cycle regulation by checkpoints. Methods Mol Biol 2014; 1170:29-40.
40. Otto T, Sicinski P. Cell cycle proteins as promising targets in cancer therapy. Nat Rev Cancer 2017; 17(2):93-115.
41. Li CJ, Tsang SF, Tsai CH, *et al*. *Momordica charantia* extract induces apoptosis in human cancer cells through caspase- and mitochondria-dependent pathways. Evid Based Complement Altern Med 2012; 2012, :261971.