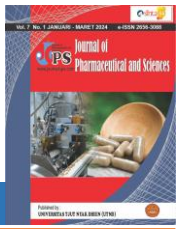
Journal of Pharmaceutical and Sciences
Electronic ISSN: 2656-3088 DOI: https://doi.org/10.36490/journal-jps.com
Homepage: https://journal-jps.com
ORIGINAL ARTICLE JPS. 2025, 8(2), 706-715



Cytotoxic Potential of Red Ginger Rhizome Extract (*Zingiber officinale var. rubrum*) Against 4T1 Breast Cancer Cells

Potensi Sitotoksik Ekstrak Rizoma Jahe Merah (*Zingiber officinale* var. rubrum) terhadap Sel Kanker Payudara 4T1

Nadia Dwi Oktaviani [a], Maryati [a]*

Department of pharmacy, Faculty of pharmacy, University Muhammadiyah Surakarta, Sukoharjo, Solo, Indonesia.

Corresponding Author: maryati@ums.ac.id

Abstract

Background: 4T1 breast cancer is a type of Triple-Negative murine breast cancer (TNBC) that has a strong ability to metastasise. Plant metabolite compounds are one of the strategies that must be explored to develop breast cancer treatments. Red ginger rhizome is one of the candidate plants that have anticancer activity. Previously, cytotoxic studies have been conducted on Widr and HeLa cancer cells. However, research on breast cancer cells is still lacking, so it needs to be developed. **Materials and Methods:** This study aims to determine the potential cytotoxicity in vitro using the MTT test. The red ginger rhizome extract is obtained by maceration using 96% ethanol solvent, which is then analysed for secondary metabolite compounds. **Results:** The results showed that red ginger rhizome extract contains flavonoids, phenolic, alkaloid, saponin, and tannin compounds. While in the cytotoxic test, red ginger rhizome extract and doxorubicin have good cytotoxic potential with IC50 values of 69.86 µg/ml and 0.4 µg/ml, respectively. The red ginger rhizome extract and doxorubicin are classified as active and highly active cytotoxics. **Conclusion:** It can be concluded that red ginger rhizome extract shows cytotoxic potential as a therapeutic candidate that can inhibit the growth of 4T1 breast cancer cells by 50%. Therefore, red ginger rhizome extract has the potential to be further developed as a chemotherapeutic agent.

Keywords: Zingiber officinale var. rubrum, Cytotoxic, 4T1 Cell, MTT assay

Abstrak

Latar Belakang: Kanker payudara 4T1 merupakan salah satu jenis kanker payudara murine Triple-Negative (TNBC) yang memiliki kemampuan metastasis yang kuat. Senyawa metabolit tanaman merupakan salah satu strategi yang perlu dieksplorasi untuk mengembangkan pengobatan kanker payudara. Rimpang jahe merah merupakan salah satu kandidat tanaman yang memiliki aktivitas antikanker. Sebelumnya, penelitian sitotoksik telah dilakukan terhadap sel kanker Widr dan Hela. Namun, penelitian terhadap sel kanker payudara masih kurang, sehingga perlu dikembangkan. **Bahan dan Metode:** Penelitian ini bertujuan untuk mengetahui potensi sitotoksik secara in-vitro menggunakan uji MTT. Metode untuk memperoleh ekstrak rimpang jahe merah adalah dengan cara maserasi menggunakan pelarut etanol 96% yang kemudian dianalisis senyawa metabolit sekundernya. **Hasil:** Hasil penelitian menunjukkan bahwa ekstrak rimpang jahe merah mengandung senyawa flavonoid, fenolik, alkaloid, saponin dan tanin. Sedangkan pada uji sitotoksik, ekstrak rimpang jahe merah dan doksorubisin memiliki potensi sitotoksik yang baik dengan nilai IC50 masing-masing sebesar 69,86 µg/mL dan 0,4 µg/mL. Ekstrak rimpang jahe merah dan doksorubisin tergolong sitotoksik aktif dan sangat aktif. **Kesimpulan:** Dapat disimpulkan bahwa ekstrak rimpang jahe merah menunjukkan potensi sitotoksik sebagai kandidat terapi yang mampu menghambat pertumbuhan sel kanker payudara 4T1 hingga 50%. Oleh karena itu, ekstrak rimpang jahe merah berpotensi untuk dikembangkan lebih lanjut sebagai agen kemoterapi.

Kata Kunci: Zingiber officinale var. rubrum, Sitotoksik, Sel 4T1, MTT assay

https://doi.org/10.36490/journal-jps.com.v8i2.796

Article History:
Received: 17/01/2025,
Revised: 20/03/2025,
Accepted: 27/03/2025,
Available Online: 25/04/2025.

QR access this Article

Introduction

Cancer remains a problem in Indonesia and is the leading cause of death worldwide. Cancer is a condition in which the growth of normal cells in the body is disrupted, causing organ damage. According to global statistics, cancer became a persistent problem in 2018, with more than 15 million cases and 9 million deaths [1]. The spread of the disease characterises cancer in several parts of the human body that cannot be treated or stopped; therefore, it will continue to spread [2]. According to *Global Cancer Observatory* (GLOBOCAN) data in 2020, there were approximately 396,914 cancer cases in Indonesia, and breast cancer cases ranked first with 65,858 patients, or with a percentage of 16.6% [3]. Cancer with malignancy in breast tissue originating from the epithelial ducts or lobules is referred to as breast cancer. The incidence of breast cancer continues to increase gradually worldwide. According to the World *Health Organisation* (WHO), in 2014, there was a 21.4% increase in the number of women in Indonesia due to breast cancer. 4T1 breast cancer is a murine breast cancer cell type with triple-negative immunological characteristics and a strong metastatic ability. This type of cancer has affected 12-17% of the world population. Compared with other breast cancers, Triple-Negative Cancer (TNBC) has a higher aggressiveness and recurrence rate [3].

According to the cancer mortality rates, chemotherapy is still unable to overcome cancer. Doxorubicin is a chemotherapeutic agent that is commonly used to treat breast cancer. However, doxorubicin has several disadvantages, including damage to non-cancerous cells, severe side effects, and the development of resistance to the medication. The common side effects include cardiomyopathy, congestive heart failure, and immunosuppression. [4]. Therefore, it is necessary to develop new strategies for the treatment of breast cancer. Several studies have been conducted to identify anticancer agents from natural products to prevent and treat cancer. Many natural ingredients can be utilised, one of which is found in Indonesia, a tropical country with biodiversity that has great potential for obtaining alternative medicines for anticancer [5].

Ginger is the most commonly used herbal plant in Asian countries and has been used for generations in various treatments since ancient times. Red ginger rhizome (*Zingiber officinale var. rubrum*) is a ginger variety that differs from other types, especially in its layered rhizomes and orange to red colour. [6]. Red ginger rhizome (*Zingiber officinale var. rubrum*) is known to have a more favourable effect than some other types of ginger, because it has an emetic impact that is indispensable for the use of cancer drugs [7]. Red ginger rhizomes contain 6-gingerol and 6-shogaol compounds, which are known to have anticancer effects. [8]. According to recent research, 6-gingerol on MDA-MB-231 breast cancer cells can inhibit the cell cycle, proliferation, and induce apoptosis. Meanwhile, 6-shogaol has a significant anticancer effect on cancer cells by inhibiting cell survival and inducing apoptosis through STAT3 activity [9;10]. Based on the isolation results, the active compounds 6-shogaol and 10-gingerol from red ginger rhizomes obtained IC_{50} values of 29.18 μM and 25.68-37.52 μM, respectively [11]. According to other studies conducted in vitro and in vivo, essential oils and extracts of red ginger rhizomes can inhibit the growth of cervical cancer cells. However, research on their cytotoxic effects, especially against breast cancer cells, is still minimal. Therefore, chemotherapeutic agents with natural ingredients are promising for overcoming cancer treatment. Thus, this study aimed to determine the cytotoxic potential of red rhizomes in 4T1 breast cancer cells.

Electronic ISSN : 2656-3088
Homepage: https://www.journal-jps.com

Experimental Section

Materials and Apparatus

Red-ginger rhizome powder was obtained from the Centre for Research and Development of Medicinal Plants and Traditional Medicines (BPTO), Tawangmangu, Indonesia. The dried red ginger rhizome powder was extracted with 96% ethanol. The results were evaporated using a rotary evaporator to obtain the red ginger rhizome extract residue. The extract was dissolved in dimethyl sulfoxide before being used as a treatment sample. Cultured 4T1 cells were obtained from the Biotechnology Laboratory of the Faculty of Pharmacy, University of Muhammadiyah Surakarta. 4T1 cells were routinely cultured in DMEM supplemented with 10% FBS (Sigma-Aldrich, USA), Fungizone (Gibco), and 1% Penicillin Streptomycin (Gibco) in an incubator. DMEM media, Sodium Bicarbonate and Hepes, Cell Culture Media, MTT (Sigma), Stopper Reagent, Sodium Solution (SDS), Doxorubicin, Phosphate Buffer Saline (PBS), and trypsin-EDTA.

The authors This research uses tools such as a Buchner funnel, analytical balance (Ohaus), rotary evaporator, water bath, micropipette 20, 200, 1000 µL, incubator (Binder, type Coinkubator2), autoclave, ELISA reader (Bio-Tech), hemocytometer (Asistant), Cytotoxic Safety Cabinet (ESCO, type cytoculture), 96 healthy plate, petri dish, Eppendorf, conical tube, pipette, test tube, volumetric flask, and inverted microscope.

Sample preparation of red ginger rhizome extract

A 500-gram sample of ginger rhizome powder was extracted with 5 L of 96% solvent and macerated for 72 h. The filtrate was concentrated using a rotary evaporator and a water bath temperature of 50 °C to obtain a thick extract [12].

Phytochemical screening

Phytochemical screening was performed to identify the compounds present in the sample. The compounds detected are flavonoids, phenolics, tannins, saponins, and alkaloids. Each test consisted of 1 g of red ginger rhizome extract and 2 ml of 96% ethanol, after which 1 ml was collected. The flavonoid test was performed by adding five drops of 0.5 M NaOH and shaking. Positive indicators were indicated by changes in colour to yellow, brown, or orange. Alkaloid test with as many as two drops of Dragendorff reagent. Positive results were obtained in the presence of an orange or orange-brown clumpy precipitate. The saponin test sample was mixed with distilled water, boiled until hot, dripped with 2N HCl, shaken, and allowed to stand for 15 min. A positive reaction indicated the formation of a stable froth for ± 10 min. For the tannin test, a few drops of $FeCl_3$ 1%. A blackish-green or blackish-blue colour change indicates a positive tannin compound. [13]. The phenolic test was dripped with 2-3 iron (III) ($FeCl_3$) 5%. A positive reaction is indicated if it produces a blue-black color.

Cytotoxic test (MTT assay)

4T1 cells were cultured in 96-well plates measuring 8×10^3 cells/well and then incubated until 80% confluence was reached. Various concentrations of red ginger rhizome extract (50, 100, 200, 400, 800 µg/ml) and doxorubicin (0.16, 0.3, 0.6, 1.25, 2.5, 5 µg/ml) were added to the wells, then kept in the incubator overnight. After that, MTT solution was added, the mixture was incubated again for 2-4 hours, and 10% SDS 0.01 N HCL solution was added to measure the absorbance using an ELISA reader (Bio-Rad) [15;16].

Data analysis

The IC_{50} value was used as a cytotoxicity parameter. The IC_{50} value is the concentration that inhibits 50% of cell proliferation. Cell viability was calculated using the following equation. [16]:[17]

$$\%\ Cell\ Viabillity = \frac{treatmentabsorbance - controlmediaabsorbance}{controlcellabsorbance - controlmediaabsorbance} \times 100\% \quad (1)$$

Data analysis was performed using linear regression between log concentration and percentage of live cells. IC_{50} value by entering the value of 50% in y and finding the antilog of the x value in the equation y = bx + a

Electronic ISSN : 2656-3088
Homepage: https://www.journal-jps.com

Results and Discussion

This chapter might be divided into several parts (at least three subheadings). If the section is divided, a general explanation will be provided before the subheadings. The headings shall be typed in bold capitals in Palatino Linotype with a font size 10. The subheadings shall be typed in bold in Palatino Linotype with a font size 9. The first letter of subheadings must be capitalised.

Red Ginger Rhizome Extract

Determination was carried out in this study to ensure the true identity of the red ginger rhizome plants used as the test material and to avoid errors in plant collection. This determination was made at the Physics Laboratory of UPH Yankestrad-RSUD, Dr. Sardjito Tawangmangu. The results showed that the red ginger rhizome plant sample came from the species *Zingiber officinale var. rubrum*. To obtain extracts from red ginger rhizomes, it was necessary to extract them by maceration with 96% solvent. The most common extraction method is the maceration method, which involves soaking the plant powder in a suitable solvent in a closed container and storing it at room temperature [18]. 96% ethanol was chosen because it is universal, non-toxic, has good sensitivity, and can detect semi-polar, polar, and non-polar compounds. In addition, 96% ethanol can penetrate the sample's cell wall because this solvent is easily absorbed, resulting in a concentrated ethanol extract [19]. The results of the maceration obtained from red ginger rhizome powder are presented in Table 1. Red ginger rhizome extract produced 119.88 grams with a dark brown colour, spicy taste, distinctive odour of red ginger rhizome, and thick texture. The yield obtained was 23%, which fulfils the requirements of the extract, which is the requirement for a good extract yield of more than 10% [20].

Table 1. Extract yield of red ginger rhizome (*Zingiber officinale var. rubrum*).

Sample	Powder weight (g)	Extract Weight (gr)	Randemen (%)
Red Ginger Rhizome Extract	500	119,88	23%

Physicochemical screening of red ginger rhizome extracts

Phytochemical screening was performed to detect the active compounds contained in the plants. This study was conducted using red ginger rhizome extract. Based on the results of phytochemical screening, the red ginger rhizome extract contained alkaloids, flavonoids, saponins, tannins, and phenolic compounds, as shown in the following table:

Table 2. Phytochemical screening of red ginger rhizome (*Zingiber officinale var. rubrum*).

Sample	Test parameters	Color change	Hasil
Red Ginger Rhizome Extract	Alkaloids	The precipitate is orange-brown or orange in colour	+
	Flavanoids	Orange, yellow, or brown colour	+
	Saponin	The presence of foam is stable for ± 10 minutes.	+
	Phenolic	Blackish blue	+
	Tanin	Blackish green or blackish blue	+

The results of the research that have been obtained are in line with previous research, namely, red ginger rhizome extract contains phytochemical compounds such as saponins, alkaloids, tannins, flavonoids, glycosides, starch, and terpenoids [21]. In previous studies, red ginger rhizomes contained 169 compounds

Electronic ISSN : 2656-3088
Homepage: https://www.journal-jps.com

such as vanilloids, monoterpenes, sesquiterpenes, diterpenes, flavonoids, and amino acids [22]. In addition, it has been proven that red ginger rhizomes contain phenolic compounds, such as gingerol and shogaol. The inhibitory effects of gingerol and shogaol are known to reduce chemotherapy-induced nausea and vomiting. Gingerol and shogol are thought to exert antiemetic effects by binding to serotonin binding sites through their action on the 5-HT3 receptor ion channel complex [23]. Ghasemzadeh et al. (2010) reported that the total amount of phenolics and flavonoids in red ginger was higher than that in normal ginger [24].

Cytotoxic test

This cytotoxicity test was conducted to determine the potential cytotoxicity of red ginger rhizome extract and doxorubicin against 4T1 breast cancer cells with the IC_{50} value test parameter, which can inhibit cell growth by 50%. 4T1 cells were chosen because of the few previous studies on red ginger rhizome extract. In addition, this type of cancer has a higher aggressiveness and recurrence rate than other types of breast cancer. [3]. Previous studies have shown that red ginger rhizome extract has a cytotoxic effect with IC_{50} values of 68 µg/ml and 65 µg/ml for 24 and 48 h, respectively, in WiDr cancer cells. Another study reported that the ethanol extract of red ginger rhizome has cytotoxic activity on HeLa cancer cells with an IC_{50} value of 104.22 ± 6.18 µg/ml. [7]. Based on the results of research on breast cancer cells, 4T1 red ginger rhizome extract and doxorubicin showed cytotoxic activity, with the increasing concentration of the value of % cell viability decreases (Figure 1. A). The treated cells showed cytotoxic effects, with changes in cell morphology and decreased cell viability. Cells alive after the sample treatment showed shrinkage (Figure 1. B).

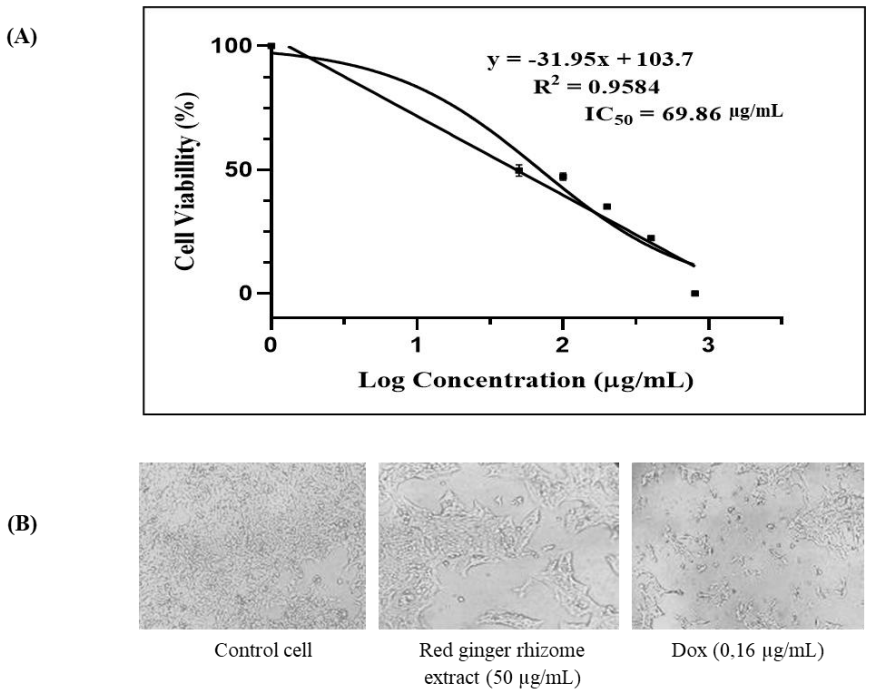


Figure 1. Effect of red ginger rhizome extract and morphological changes in 4T1 breast cancer cells. (**A**) The effect of red ginger rhizome extract on 4T1 breast cancer cells was determined by MTT assay for 24 hours, as described in Materials and Methods. (**B**) Morphological changes in 4T1 breast cancer cells were observed under an inverted microscope at 100x magnification. Complete cell control and morphological changes (cell shrinkage into a sphere)

Cell viability occurs with the formation of purple formazan due to the reduction of tetrazolium compounds (MTT), which were previously yellow. This happens because the MTT compound is absorbed by living cells, and the enzyme succinate reductase reduces tetrazolium salts in the mitochondria into purple formazan crystals that are insoluble in water and settle in the cell. The formation of purple formazan indicated the presence of living cells in the wells (cell viability). The cytotoxic activity of the extract against cancer cells was classified as very active if the IC_{50} value was <10 µg/ml, active category was between 10-100 µg/ml, and sufficient if the IC_{50} value was 100-500 µg/ml [15]. The IC_{50} value was determined through probit analysis

Electronic ISSN : 2656-3088
Homepage: https://www.journal-jps.com

based on the graph of log concentration versus the percentage of live cells (cell viability) (Figure 1. A) shows that red ginger rhizome extract has potential as an anticancer with an active cytotoxic category obtained IC_{50} value of 69.86 µg/ml. This indicates that the red ginger rhizome extract can inhibit the growth of 4T1 breast cancer cells by 50%. Doxorubicin has a cytotoxic effect with an IC_{50} value of 0.4 µg/ml. This study aligns with previous research showing that doxorubicin is a chemotherapeutic agent with potent activity against 4T1 breast cancer cells (TNBC) with a very active IC_{50} value of 1.2 µg/ml. Therefore, doxorubicin in this study had an IC_{50} value of less than 10 µg/ml, indicating a powerful cytotoxic effect. The long-term use of doxorubicin can result in severe side effects, such as toxicity to normal cells and cancer resistance. Therefore, this study will produce a more effective treatment by identifying a natural medicine. The red ginger rhizome extract was found to counteract the growth of 4T1 breast cancer cells, which has an active cytotoxic effect. Therefore, further studies are needed to evaluate the underlying molecular and physiological mechanisms.

Several molecular mechanisms that may occur due to the effect of red ginger rhizome extract on 4T1 cancer cells include the induction of apoptosis, cell cycle arrest at the G0/G1 and G2/M phases, and effects on the P53 and STAT3 pathways.

Induction of Apoptosis in 4T1 Cancer Cells

The induction of apoptosis is increasingly recognised as a critical mechanism through which red ginger extract, derived from Zingiber officinale var. rubrum, exerts its cytotoxic effects in cancer cell lines. Multiple studies indicate that the apoptotic process can specifically engage the intrinsic pathway involving caspases, particularly caspase-9 and caspase-3. Following mitochondrial stress, cytochrome c is released into the cytoplasm, activating these caspases, leading to a cascade of proteolytic events that culminate in cell death. [25,26]. These findings underscore the importance of the mitochondria in red ginger's action against cancerous cells, which is consistent with reports on other natural compounds inducing apoptosis via similar mechanisms. [27,28].

Additionally, regulating Bcl-2 and Bax proteins is integral to these processes. Red ginger extract has been shown to significantly modify the expression levels of Bcl-2 (an anti-apoptotic protein) and Bax (a pro-apoptotic protein). Increased levels of Bax promote apoptosis by facilitating the release of apoptotic factors, further activating caspase pathways. [28]. Specifically, the modulation of these proteins has been linked to the extract's ability to enhance pro-apoptotic signals while diminishing anti-apoptotic ones, suggesting that red ginger could serve as a promising adjunct in cancer therapy. [29].

It is critical to analyse further the changes in Bax, Bcl-2, and caspase-3 expression within cancer cell lines. Notable studies corroborate that red ginger's bioactive components, like gingerol and related compounds, effectively target these pathways, thereby exerting anti-cancer effects through apoptosis. [27,28]. Such mechanistic insights could pave the way for future therapeutic interventions leveraging red ginger extract's potential. [29]. Furthermore, the antioxidant properties of red ginger are also suggested to play a role in modulating oxidative stress-related pathways associated with apoptosis, adding another layer of complexity to its mechanism of action. [30].

Cell Cycle Arrest at the G0/G1 and G2/M Phases

Red ginger extract has been increasingly recognised for its potential to induce cell cycle arrest, particularly at the G0/G1 and G2/M phases. In the G0/G1 phase, cell cycle arrest regulates key proteins such as cyclin D1 and cyclin-dependent kinases (CDK4/6). Research has shown that red ginger extract can effectively downregulate the expression of cyclin D1, while upregulating the expression of the cyclin-dependent kinase inhibitors p21 and p27, which serve to inhibit CDK activity and subsequently impede the transition from the G1 phase to the S phase of the cell cycle. [31,32]. This regulatory mechanism is crucial as CDKS play significant roles in cell cycle progression, and their inhibition can directly halt cell division.

Further analysis confirms that red ginger extract influences cell cycle dynamics at the G2/M phase. The transition from G2 to M phase typically relies on activating cyclin B and CDK1. Studies have demonstrated that red ginger extract leads to the downregulation of cyclin B and CDK1, which is essential for this transition. [33,34]. Additionally, decreased expression of tumour suppressor protein p53 and increased levels of p21 contribute to this cell cycle arrest, highlighting a multifaceted approach whereby both the upregulation of cyclin inhibitors and the downregulation of cyclins and CDKS play integral roles in cell cycle regulation. [35,36].

Electronic ISSN : 2656-3088
Homepage: https://www.journal-jps.com

Further investigation into the expression levels of these proteins in 4T1 cells treated with red ginger extract can provide valuable insights into its mechanisms of action on cell cycle regulation. Specifically, measuring the changes in cyclin D1, p53, and p21 expression will be crucial in understanding how red ginger extract mediates its effects on cell cycle arrest behaviour [32,34]. Notably, several studies emphasize the significance p21 in signaling pathways that can lead to either cell cycle arrest or apoptosis, thus underscoring the potential therapeutic value of red ginger extract in cancer treatment [37].

Effect on the P53 and STAT3 Pathways

The regulatory role of p53 in cellular response to DNA damage is pivotal, particularly in cancer biology, where its expression levels can influence cell cycle arrest and apoptosis. P53, often labeled the "guardian of the genome," can activate the expression of key cell cycle inhibitors such as p21 and p27 in response to stressors, including oxidative stress and DNA damage. Evidence suggests that ginger extracts can influence p53 expression, with studies indicating that treatment with ginger can impact the levels of anti-apoptotic proteins like Bcl-2 and induce apoptotic pathways through activating pro-apoptotic factors such as Bax.[38,39].

Research indicates that red ginger extract may induce oxidative stress, potentially triggering DNA damage and activating the p53 pathway in cancer cells. It is hypothesized that exposure of 4T1 breast cancer cells to red ginger extract will result in observable changes in p53 expression, reflecting p53's role in mediating cellular responses to such stresses. A decrease in p53 expression in these cancer cells may indicate evasion of cellular control mechanisms, highlighting the importance of evaluating p53 levels to assess the potential effectiveness of red ginger extract in combating tumor growth. [38,40].

Another significant pathway associated with cancer progression is the STAT3 signaling pathway. STAT3 is a transcription factor frequently activated in various cancers, including breast cancer, contributing to cell proliferation and survival. Red ginger extract's inhibition of this pathway represents a potential therapeutic strategy. Specifically, the phytochemical components in ginger, such as gingerol, have been documented to inhibit STAT3 activation and downregulate the expression of genes involved in cell proliferation and survival. [40,41]This inhibitory effect of red ginger on STAT3 phosphorylation and activation could further reinforce its potential as an anticancer agent by not only activating pro-apoptotic signals via the p53 pathway but also by mitigating pathways that promote cell survival, thereby enhancing the cytotoxic effects on cancer cells.

Conclusions

Based on these findings, red ginger rhizome extract can inhibit the growth of 4T1 breast cancer cells by 50% with an IC_{50} value of 69.86 µg/mL, equivalent to an active cytotoxicant of 10-100 µg/mL. Therefore, the red ginger rhizome extract is a natural ingredient that can be further developed as a chemotherapeutic agent.

Conflict of Interest

The authors declare that they have no conflict of interest regarding the publication of this article. They confirm that no financial, personal, or professional relationships or affiliations could be perceived as influencing the content or results presented in this work.

Acknowledgment

The researcher would like to thank the Research Institute of the Faculty of Pharmacy, Universitas Muhammadiyah Surakarta, for facilitating this research.

References

[1] Jiang N, Dai Q, Su X, Fu J, Feng X, Peng J. Role of PI3K/AKT pathway in cancer: the framework of malignant behavior. Vol. 47. Springer Netherlands; 2020. https://doi.org/10.1007/s11033-020-05435-1.

Electronic ISSN : 2656-3088
Homepage: https://www.journal-jps.com

[2] Shafirany MZ, Indawati I, Sulastri L, Sadino A, Kusumawati AH, Alkandahri MY. Antioxidant Activity of Red and Purple Rosella Flower Petals Extract (Hibiscus sabdariffa L.). Journal of Pharmaceutical Research International 2021;33:186 92. https://doi.org/10.9734/jpri/2021/v33i46b32931.

[3] Dewi MP, Indrayudha P, Maryati, Saifudin A, Muflihah CH. Cytotoxic Activity and Antiproliferation of Soursop Seed Protein (Annona muricata) Against 4T1 Cells. Vol. 3. Atlantis Press International BV; 2023. https://doi.org/10.2991/978-94-6463-050-3_31.

[4] Laksmiani NPL, Susidarti RA, Meiyanto E. Brazilein Increased Cytotoxic Activity of Doxorubicin on MCF-7/DOX Cells. Indonesian Journal of Cancer Chemoprevention 2017;6:58. https://doi.org/10.14499/indonesianjcanchemoprev6iss2pp58-63.

[5] Dira O. Cytotoxic Tests On Rhizome Plants In Indonesia Used As An Anticancer: A Review. … Journal of Pharmaceutical Research and … 2020;5:492 9. https://doi.org/10.35629/7781-0502492499.

[6] Anggraini K, Setyaningrum DAW, Wulansari L, Andayani HT, Shihran LP, Fauziyyah I. Effect of Extraction Solvent on Extraction Yield, Cytotoxic Activity and Bioactive Compound in Zingiber officinale Roscoe var rubrum. Vol. 1. Atlantis Press International BV; 2023. https://doi.org/10.2991/978-94-6463-284-2_86.

[7] Rofida S, Nurani LH, Utami D, Edityaningrum CA. Cytotoxicity of Zingiber officinale var. rubrum on HeLa cells and prediction of anti-proliferative activity via the JAK2 / STAT3 and hedgehog pathways using a molecular docking approach 2023;13:291 305. https://doi.org/10.12928/pharmaciana.v13i3.27482.

[8] Sarmoko, Solihati I, Setyono J, Ekowati H, Fadlan A. Zingiber officinale Var. rubrum extract increases the cytotoxic activity of 5-fluorouracil in colon adenocarcinoma Widr cells. Indonesian Journal of Pharmacy 2020;31:266 72. https://doi.org/10.22146/ijp.859.

[9] Sp N, Kang DY, Lee JM, Bae SW, Jang KJ. Potential antitumor effects of 6-gingerol in p53-dependent mitochondrial apoptosis and inhibition of tumor sphere formation in breast cancer cells. International Journal of Molecular Sciences 2021;22. https://doi.org/10.3390/ijms22094660.

[10] Jia Y, Li X, Meng X, Lei J, Xia Y, Yu L. Anticancer perspective of 6-shogaol: anticancer properties, mechanism of action, synergism and delivery system. Chinese Medicine (United Kingdom) 2023;18:1 21. https://doi.org/10.1186/s13020-023-00839-0.

[11] Zhang M, Zhao R, Wang D, Wang L, Zhang Q, Wei S, et al. Ginger (Zingiber officinale Rosc.) and its bioactive components are potential resources for health-beneficial agents. Phytotherapy Research 2021;35:711 42. https://doi.org/10.1002/ptr.6858.

[12] Dianasari D, Puspitasari E, Ningsih IY, Triatmoko B, Nasititi FK. Potensi Ekstrak Etanol dan Fraksi-Fraksinya Dari Tiga Varietas Jahe Sebagai Agen Antibakteri Terhadap Staphylococcus aureus. Pharmacon: Jurnal Farmasi Indonesia 2020;17:9–16. https://doi.org/10.23917/pharmacon.v17i1.9226.

[13] Iskandar D, Ananda D, Putri M, Hidayani R. Uji Kualitatif Senyawa Metabolit Sekunder Ekstrak Daun Malapari (Pongamia pinnata L. Pierre) Pada Pelarut Etanol dan n-Heksana Sebagai Kandidat Sunscreen. Jurnal Ilmiah Pendidikan Dasar 2024;6:107–14. https://doi.org/10.37216/badaa.v6i1.1400.

[14] Husnaa U, Laksmiani NPL, Susidarti RA, Meiyanto E. Ethyl Acetate Fraction of Caesalpinia sappan L. Enhances Cisplatin's Cytotoxicity on HeLa Cells via G1 and S Arrest through p53 Expression. Indonesian Journal of Cancer Chemoprevention 2017;8:51. https://doi.org/10.14499/indonesianjcanchemoprev8iss2pp51-60.

[15] Fatmawati KI, Indrayudha P, Maryati, Saifudin A, Muflihah CH. Cytotoxic Activity of Melinjo Seed Protein (Gnetum Gnemon L.) Against 4T1 Cells and HeLa Cells, and Antiproliferation Test on 4T1 Cells. Vol. 3. Atlantis Press International BV; 2023. https://doi.org/10.2991/978-94-6463-050-3_32.

[16] Pamungkas AR, Indrayudha P. Aktivitas Sitotoksik Ekstrak Etanol, Fraksi Etanol-Air, Etil Asetat serta N-Heksana Buah Pare (Momordica charantia) pada Sel MCF-7 secara In-Vitro. Pharmacon: Jurnal Farmasi Indonesia 2019;16:73–82. https://doi.org/10.23917/pharmacon.v16i2.9049.

[17] Merlin, Runtuwene M, Kamu V. Kandungan Total Fenolik Dan Uji Toksisitas Daun Muharang Bawine (Dendrophthoe falcate (Lf) Etinggsh) Dengan Metode BLST (Brine Shrimp Lethality Test). Chemistry Progress 2020;13. https://doi.org/10.35799/cp.13.1.2020.28830.

[18] Badaring DR, Sari SPM, Nurhabiba S, Wulan W, Lembang SAR. Uji Ekstrak Daun Maja (Aegle marmelos L.) terhadap Pertumbuhan Bakteri Escherichia coli dan Staphylococcus aureus Indonesian Journal of Fundamental Sciences (IJFS). Indonesian Journal of Fundamental Sciences 2020;6:16–26.

Electronic ISSN : 2656-3088
Homepage: https://www.journal-jps.com

[19] Qonitah F, Ariastuti R, Pratiwi M, Wuri NA. Skrinning Fitokimia Ekstrak Etanol Daun Jeruk Purut (Citrus hystrix) Dari Kabupaten Klaten. Gema 2022;34:47–51.
[20] Oktarina T, Indrayudha P, Munawaroh R. Cytotoxicity Test of N-Hexane, Ethyl Acetate, And Water Fractions From The Ethanol Extract Of Senduduk Leaves (Melastoma malabathricum L.) on MCF-7 Cells Using The MTT Assay Method. Medical Sains : Jurnal Ilmiah Kefarmasian 2024;9:355–62. https://doi.org/10.37874/ms.v9i1.1119.
[21] Novianti, Asmariyah, Suriyati, Wirahmi N. Physical Evaluation of Red Ginger Extract (Zingiber Officinale Rosc. Var. Rubrum) Cream of 10% and 20% Concentration To Reduce Low Back Pain for Pregnant Women in Second and Third of Trimesters. International Journal of Applied Pharmaceutics 2022;14:4 7. https://doi.org/10.22159/ijap.2022.v14s1.02.
[22] Zhang S, Kou X, Zhao H, Mak KK, Balijepalli MK, Pichika MR. Zingiber officinale var. rubrum: Red Ginger's Medicinal Uses. Molecules 2022;27. https://doi.org/10.3390/molecules27030775.
[23] Choi J, Lee J, Kim K, Choi HK, Lee SA, Lee HJ. Effects of Ginger Intake on Chemotherapy-Induced Nausea and Vomiting: A Systematic Review of Randomized Clinical Trials. Nutrients 2022;14:1–17. https://doi.org/10.3390/nu14234982.
[24] Ghasemzadeh A, Jaafar HZE, Rahmat A. Antioxidant activities, total phenolics and flavonoids content in two varieties of malaysia young ginger (Zingiber officinale Roscoe). Molecules 2010;15:4324–33. https://doi.org/10.3390/molecules15064324.
[25] Wee LH, Morad NA, Aan GJ, Makpol S, Ngah WZW, Yusof YAM. Mechanism of chemoprevention against colon cancer cells using combined Gelam honey and Ginger extract via mTOR and Wnt/β-catenin pathways. Asian Pacific Journal of Cancer Prevention 2015;16:6549 56.
[26] Tahir AA, Sani NFA, Murad NA, Makpol S, Ngah WZW, Yusof YAM. Combined ginger extract & Gelam honey modulate Ras/ERK and PI3K/AKT pathway genes in colon cancer HT29 cells. Nutr J 2015;14:1 10.
[27] Luo L, Chen Y, Ma Q, Huang Y, Hong T, Shu K, et al. Exploring the mechanism of an active ingredient of ginger, dihydrocapsaicin, on triple negative breast cancer based on network pharmacology and in vitro experiments. Oncol Lett 2023;25:195.
[28] Huang P, Zhou P, Liang Y, Wu J, Wu G, Xu R, et al. Exploring the molecular targets and mechanisms of [10]-Gingerol for treating triple-negative breast cancer using bioinformatics approaches, molecular docking, and in vivo experiments. Transl Cancer Res 2021;10:4680.
[29] Rasmussen A, Murphy K, Hoskin DW. 10-Gingerol inhibits ovarian cancer cell growth by inducing G2 arrest. Adv Pharm Bull 2019;9:685.
[30] Febri Sari T, Trisnadi S, Isradji I. The Effect of Red Ginger Extract on Tnf-α and Malondialdehyde Serum Level. International Journal of Multidisciplinary Research and Analysis 2022;05:2643 9875. https://doi.org/10.47191/ijmra/v5-i12-06.
[31] Putra ED, Nazliniwaty N, Syafruddin S, Nerdy N. Component analysis of white ginger (Zingiber officinale Roscoe) extract and red ginger (Zingiber officinale Rubra) extract. 2021.
[32] Zhong Y, Krisanapun C, Lee S-H, Nualsanit T, Sams C, Peungvicha P, et al. Molecular targets of apigenin in colorectal cancer cells: Involvement of p21, NAG-1, and p53. Eur J Cancer 2010;46:3365–74.
[33] Wang Y, Wu N, Zhang J, Wang H, Men X. MiR-153-5p enhances the sensitivity of triple-negative breast cancer cells to paclitaxel by inducing G2M phase arrest. Onco Targets Ther 2020:4089 97.
[34] Li W, Xie L, Chen Z, Zhu Y, Sun Y, Miao Y, et al. Cantharidin, a potent and selective PP2A inhibitor, induces an oxidative stress-independent growth inhibition of pancreatic cancer cells through G2/M cell-cycle arrest and apoptosis. Cancer Sci 2010;101:1226 33.
[35] Song J, Song W, Zhang L. LncRNA RP1-85F18 6 affects osteoblast cells by regulating the cell cycle. Open Life Sci 2020;15:951 8.
[36] Huang W-W, Ko S-W, Tsai H-Y, Chung J-G, Chiang J-H, Chen K-T, et al. Cantharidin induces G2/M phase arrest and apoptosis in human colorectal cancer colo 205 cells by inhibiting CDK1 activity and caspase-dependent signaling pathways. Int J Oncol 2011;38:1067 73.
[37] Pan Z, Zhang X, Yu P, Chen X, Lu P, Li M, et al. Cinobufagin induces cell cycle arrest at the G2/M phase and promotes apoptosis in malignant melanoma cells. Front Oncol 2019;9:853.

Electronic ISSN : 2656-3088
Homepage: https://www.journal-jps.com

[38] Pashaei-Asl R, Pashaei-Asl F, Gharabaghi PM, Khodadadi K, Ebrahimi M, Ebrahimie E, et al. The inhibitory effect of ginger extract on ovarian cancer cell line: application of systems biology. Adv Pharm Bull 2017;7:241.

[39] Rastogi N, Duggal S, Singh SK, Porwal K, Srivastava VK, Maurya R, et al. Proteasome inhibition mediates p53 reactivation and anti-cancer activity of 6-gingerol in cervical cancer cells. Oncotarget 2015;6:43310.

[40] Saha A, Blando J, Silver E, Beltran L, Sessler J, DiGiovanni J. 6-Shogaol from dried ginger inhibits growth of prostate cancer cells both in vitro and in vivo through inhibition of STAT3 and NF-κB signaling. Cancer Prevention Research 2014;7:627 38.

[41] de Lima RMT, Dos Reis AC, de Menezes APM, Santos JV de O, Filho JWG de O, Ferreira JR de O, et al. Protective and therapeutic potential of ginger (Zingiber officinale) extract and [6]-gingerol in cancer: A comprehensive review. Phytotherapy Research 2018;32:1885 907.

Electronic ISSN : 2656-3088
Homepage: https://www.journal-jps.com